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SEXUAL PLANTS WITH HIGH CHROMOSOME NUMBER FROM AN INDIVIDUAL PLANT SELECTION IN A NATURAL POPULATION OF GUAYULE AND MARIOLA

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INTRODUCTION

FACULTATIVE apomixis has been shown to be the predominant method of reproduction in guayule and mariola (*Parthenium argentatum* Gray and *P. incanum* H. B. K.) (POWERS AND ROLLINS 1945). All guayule studied thus far with 54± or more chromosomes reproduces by facultative apomixis. One sexual plant has been described from a strain with mariola-like characteristics (POWERS and ROLLINS 1945). This plant has not been studied cytologically. Only 36± chromosome strains of guayule are known to be completely sexual (POWERS and ROLLINS 1945). One individual plant selection (42418) with high chromosome numbers from seed collected in a natural population of guayule and mariola by POWERS and FEDERER (POWERS 1942) appears to be completely sexual. This is of interest both from the practical and theoretical standpoint. Sexual plants are necessary in the development of new and improved strains in a breeding program. A progeny of sexual plants with varying chromosome numbers will contribute toward a better understanding of the method of reproduction in guayule and mariola. This knowledge is essential to the breeding program. The inheritance of apomixis and its relationship to polyploidy in the evolution of the group can be better understood from an analysis of data concerning these sexual polyploids.

Chromosome determinations made on the progeny of the plant from which seed collection 42418 was made showed surprisingly high chromosome numbers for sexual plants. Practically all had 80 or more chromosomes. POWERS (1942) described the original plant as more nearly approaching mariola in characteristics than guayule, but having large leaves and flowers and flower stalks like guayule. The seed was collected by POWERS and FEDERER on the individual plant basis so that it might be determined whether guayule had entered into its parentage. ROLLINS (1945) suggested that the plant must have been a hybrid between guayule and mariola. Data obtained from chromosome studies, rubber and resin determinations, seed germinations, pollen measurements, plant size measurements, leaf measurements, and progeny tests will be used as evidence of sexuality. Practical and theoretical considerations will be discussed.

MATERIALS AND METHODS

Seed from collection 42418 was germinated in the greenhouse, and 24 plants

* Now located at Salinas Junior College, Salinas, California

were placed in the field in the spring of 1943 as culture 3053. One plant died soon after being transplanted. The remaining 23 have been studied and compared. Progeny tests of all the plants have been made.

Chromosome numbers of plants in culture 3053 were determined from pollen-mother-cells by DR. A. DOROTHY BERGNER (1944). Root tip smears prepared by MEYERS' (1945) technique were used entirely for chromosome determinations of the second generation progeny (table 2).

The percentage resin and percentage rubber were obtained from a sample of each plant taken during the dormant period in February 1944. Determinations were made at the BUREAU of PLANT INDUSTRY, SOILS and AGRICULTURAL ENGINEERING RUBBER LABORATORY at Salinas, California.

Pollen size was determined by measuring 100 pollen grains from each plant (see POWERS and GARDNER 1945 for technique).

Germination tests were made by planting 1,000 seeds from each plant in soil in the greenhouse. The seed was first treated with clorox by the method reported by BENEDICT and ROBINSON (in press) and proved satisfactory for guayule.

Leaf measurements were obtained by picking ten leaves at random from each plant and measuring the length and width of the leaf and length of the petiole in centimeters. The length measurement represents the greatest length from the tip to the point of attachment of the petiole with the plant stem. The width is measured just above the first two major points. The petiole is measured from the attachment end to the place where the blade of the leaf widens abruptly. The number of points represents the total number of points on the leaf and petiole, not including the tip on the extremity of the leaf.

The height and spread is the measurement of the two-year-old plants in centimeters. The height is measured from the ground to the top of the highest leaf on the plant and the spread is the distance from one side to the other through the center of the plant.

Coefficients of variability and standard errors of the coefficients of variability were calculated from the data concerning the characters described above.

Second generation progeny of 42418 were obtained from both open and controlled pollinations of plants in the culture 3053. Three of the plants (3053–10, 14, and 19) were selfed. The same three plants were used as seed parents in crosses with 36± chromosome guayule (accession 4255), 72± chromosome guayule (42354-II), 72± chromosome commercial guayule (strain 593), and 72± chromosome mariola (43684-III). The 36± chromosome guayule is known to be sexual, both 72± chromosome strains of guayule are highly apomictic, and the mariola is also apomictic.

EXPERIMENTAL RESULTS

Progeny from Seed Collection 42418

Characteristics of both guayule and mariola can be observed in the progeny of 42418, but the mariola characters predominate, as was true of the original parent (POWERS 1942). All 23 progeny were shown to have 70 or more chromo-

somes. All except two have more than 80, and eight have 90 or more chromosomes. POWERS (1945), STEBBINS and KODANI (1944), and BERGNER (in press) have shown that higher chromosome numbers occur in guayule, but the usual number is 72± or less. The common strains of mariola also have 72± or less chromosomes.

Table 1 summarizes the data obtained from studies of the 23 plants. The average resin content is somewhat lower than that of guayule. The average percentage determined for guayule, strain 593, is 5.36 percent (KRAMER, in press). One strain of mariola from the 02 Ranch (accession 42172) has 5.78

TABLE 1

Average data and coefficients of variability for characters observed in progeny of 42418 (culture 3053).

	PERCENTAGE RESIN	PERCENTAGE RUBBER	SIZE OF POLLEN (MICRONS)	PERCENTAGE GERMINATION	LEAF LENGTH (CM)	LEAF WIDTH (CM)	PETIOLE LENGTH (CM)	POINTS ON LEAF	HEIGHT (CM)	SPREAD CM)
Average (23 plants)	4.42	.89	26.12	2.68	3.74	.66	.90	8.56	36.09	71.09
Coefficient of variability	20.87	51.45	5.01	100.12	20.18	22.40	26.71	22.20	22.30	30.64
Coefficient of variability for apomictic strain (accession 42387)	7.66	12.11			6.19	10.86	13.82	14.04	7.40	5.90
Coefficient of variability for sexual strain (accession 4255)	14.77	22.87			11.17	18.02	21.89	66.32	12.76	15.87

percent resin. Rubber content is low like that of mariola. The average rubber content determined for mariola (accession 42172) was 0.26 percent (KRAMER, in press.)

By comparison with POWERS' (1945) data, the average size of the pollen grains is significantly larger than that of 36± and 54± chromosome guayule and significantly smaller than that of 108± chromosome plants. It is not significantly different from 72± chromosome guayule. The proportion of aborted pollen grains was not greater than that reported by POWERS and GARDNER (1945) for common strains of guayule.

Seed from the plants of culture 3053 germinated poorly. The average percentage germination was only slightly lower than that of seed from three of these same plants tested earlier in the germinator. It is therefore suspected that the low germination is largely inherent in the plants and not the result of treatment. The data show that the average germination was less than three percent. This is low compared with 18 percent for a 54± chromosome, apomictic plant also showing characteristics of guayule and mariola, which was given the same treatment and planted as a check.

In all the leaf characters the progeny of 42418 resemble mariola much more closely than guayule. This is particularly striking with regard to leaf width, length of petiole, and number of points. Mariola is characterized by short petiole, narrow leaf above the first two major points, and eight to ten leaf

points, while guayule has a long petiole, wide leaf, and one to three points. The trichomes (see ROLLINS 1944) strongly resemble mariola.

The plants are large and bushy. In size and general appearance they resemble mariola more closely than guayule. However, they are more compact and not as tall as mariola of the same age growing near them in the field. In height and spread they compare favorably with the largest plants in the field.

TABLE 2

Chromosome determinations of second generation progeny of 42418 from controlled pollinations

CROSS	NUMBER OF PLANTS STUDIED	CHROMOSOME NUMBERS*
3053-19×42354 II	5	43, 82+2, 80+2, 82+2, 83+2
3053-14×42354 II	1	84+1
3053-19×593 V	1	82
3053-19×43684 III	2	80+2, 81+1
3053-14×43684 III	1	80
3053-19×4255	3	63, 62, 64
3053-14×4255	1	67+2
3053-19 Selfed	6	88+2, 89, 91, 88, 92+2, 89

* The full size chromosomes are given with the additional miniature chromosomes observed.

Date of bloom in the spring of 1945 was intermediate between guayule and mariola. Guayule (strain 593) first bloomed from April 4 to 16, mariola (accession 42172) came into bloom from June 3 to 9 while the progeny of 42418 first bloomed between April 28 and June 9. More variation was noted here than in either guayule or mariola, but the progeny of 42418 favored mariola in the late blooming character.

Variability of the characters described above was compared with that of an apomictic strain of guayule (accession 42387) which also represents an individual plant selection from the same location. The coefficients of variability (table 1) for all eight characters compared are considerably greater than those for corresponding characters in the apomictic strain. When compared with a sexual strain (accession 4255) the progeny of 42418 showed greater coefficients of variability for percentage rubber, percentage resin, leaf length, leaf width, petiole length, height and spread and a lower coefficient of variability for points on leaf. Data concerning germination and pollen size were not available for comparison.

Second Generation Progeny from Seed Collection 42418

Progeny from both open and controlled pollinations of plants in culture 3053 were studied to determine whether or not sexuality had persisted in the second generation progeny of 42418. Chromosome determinations were made on 20 different offspring from two different seed parents. Table 2 describes the crosses and lists the chromosome numbers obtained. The data show that chromosome numbers expected on the basis of complete reduction were found in all combinations studied. One plant listed has only 43 chromosomes, while

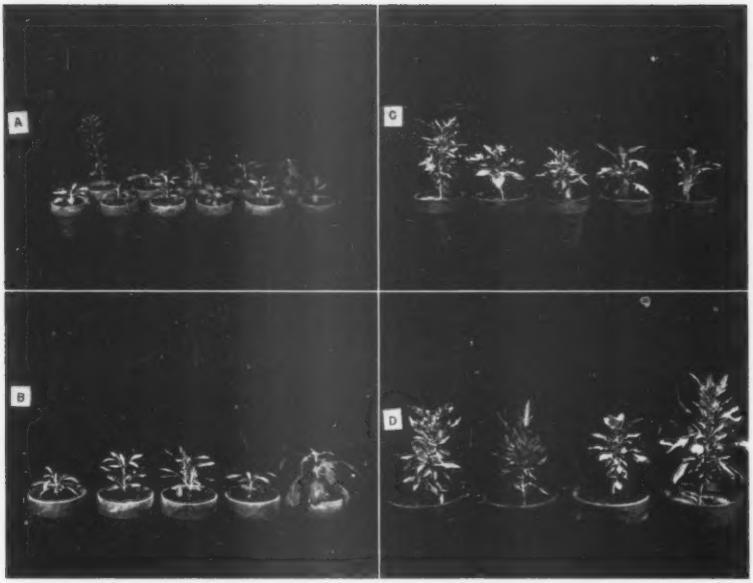

PLATE I.—Progeny of 3053–19 selected to show variation. A, self pollinated progeny; B, five plants from group shown in A, selected to show guayule and mariola characteristics; C, progeny of a cross between 3053–19 and 72± chromosome guayule; D, progeny of a cross between 3053–19 and strain 593 guayule.

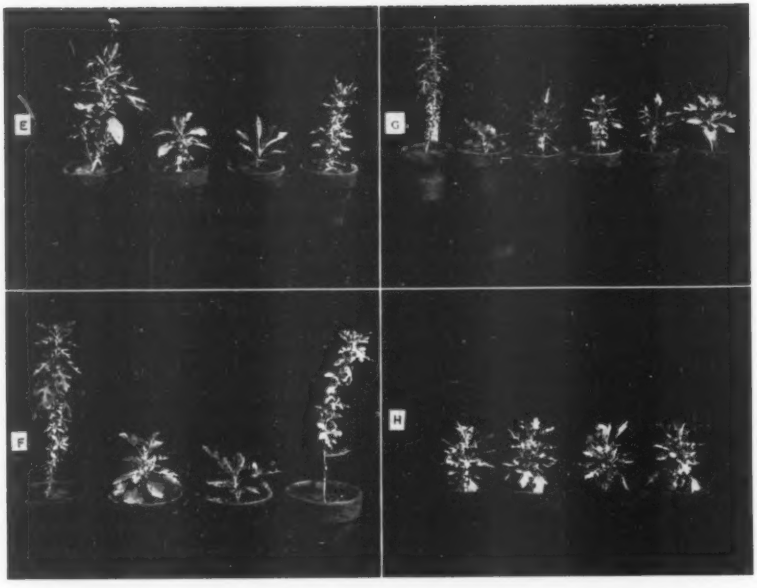

PLATE II.—Progeny of 3053–19 compared with progeny of an apomictic strain. E, progeny of cross between 3053–19 and 36± chromosome guayule; F, progeny of cross between 3053–19 and mariola; G, progeny of cross between 3053–19 and mariola compared with those of 3053–19 and guayule. The two on left show mariola characteristics; and three next show guayule characteristics; H, progeny of cross between an apomictic plant and 36± chromosome guayule. Note uniformity among apomicts (H) compared with sexuals (A-G).

all others from the same cross have 80 or more. The most obvious explanation for the low chromosome number is that a mature egg with the reduced number of chromosomes has developed without fertilization to produce this plant.

Great phenotypic variation characterizes the second generation progeny of 42418. This is true of the open pollinated offspring as well as those from controlled pollination. To illustrate the phenotypic variation, photographs (plate 1 and 2) of progeny of one seed parent (3053–19) crossed with different pollen parents are presented.

DISCUSSION

The data presented above show that polyploid plants representing the progeny of a single plant reproduce sexually. No other case of complete sexuality has been reported for a plant involving guayule above the diploid level. Facultative amphimixis has been described by POWERS (1945) for progeny of one other individual plant selection of guayule from the same location. In the case described amphimixis was shown to occur without reduction. Both reduction and fertilization occur regularly in the progeny of the plant here described. The identification of this group of sexual plants involving guayule with high chromosome number in an apomictic population has a practical as well as a theoretical significance.

The subject of apomixis as it applies here is discussed at length by GUSTAFSSON (1935, 1939), STEBBINS (1941), and POWERS (1945). The present data will be discussed with reference to the work of these authors. Terminology as defined by POWERS (1945) will be followed in this discussion.

From the practical standpoint, sexual plants are useful in the development of new and improved strains in a breeding program. Mendelian inheritance is applied to sexual material. Variation, however, does occur to a greater or lesser extent in agamic complexes. In diplospory gradations from pseudoheterotypic division which involves no synapsis to semiheterotypic division which involves synapsis in varying degrees may occur. These processes overlap each other. Pseudohomeotypic and semiheterotypic divisions may occur in the same individual. Synapsis may take place in all or none of the chromosome pairs or in any intermediate number between these extremes. Diplospory resulting from semiheterotypic division characterized by the formation of a restitution nucleus involves meiotic processes. Since crossing over does occur, apomicts of this type may give rise to new combinations of a given series of characters. Striking and predictable variation, however, is dependent on sexuality. Variation to a more limited extent may occur through somatic mutation. A sexual strain can be used to a good advantage in breeding better guayule. Apomixis also has a practical importance in the maintenance of favorable combinations once developed. To make use of these two processes in a breeding program an understanding of the inheritance of the method of reproduction is essential.

The data concerning this group of sexual plants are also interesting from a theoretical standpoint, since they add light to the problems concerning the nature and evolution of apomixis. All of the above authors point out the rela-

tionship between apomixis and polyploidy and all agree that this association is not absolute. Diploid apomictic species are known, and sexual polyploids exist in genera which also include apomicts. POWERS has suggested that polyploidy and apomixis have evolved together and that either one would have had great difficulty in becoming established or surviving without the other. The evidence indicates that the two major processes involving normal sexuality, reduction and fertilization, are largely independent. Reduction in chromosome number may occur without fertilization and fertilization may occur without reduction. The apomicts in the groups studied have undoubtedly evolved from the sexual members of the group. POWERS (1945) lists three major steps involving the change from normal sexuality to apomixis (pseudogamous diplospory) as follows: (1) failure of reduction in number of chromosomes, (2) failure of fertilization, (3) development of the egg-cell into a new individual without its being fertilized. Evidence is cited by POWERS (1945), GUSTAFSSON (1935), and STEBBINS (1941) to show that specific genes are responsible for at least some steps in the process. POWERS (1945) has pointed out the quantitative nature of inheritance of these characters. The fact that apomixis is facultative in guayule suggests the quantitative nature of inheritance of apomixis.

The data in the present article concerning sexual polyploids show that apomixis is not the result of polyploidy and suggest that it is conditioned by specific genes. No segregates for apomixis were observed in the progeny of 42418 showing that the factors for complete sexuality are well established. Since one sexual plant has been described in mariola (POWERS and ROLLINS 1945) and members of collection 42418 show evidence of hybridity between mariola and guayule (ROLLINS 1944) in their ancestry, the genes for sexuality may have come partly or entirely from the mariola side. The fact that this sexual mariola has come from an area widely separated from the location of seed collection 42418 suggests that natural hybridity has not occurred between these two strains. This does not preclude the possibility that natural hybridity could account for the case described. More extensive collections will no doubt clarify this point. Complete sexuality may occur in guayule polyploids, but no natural population reproducing by this method has been reported.

Since all other guayule collections studied with more than $36\pm$ chromosomes and all except one plant of one collection of mariola are apomictic, it seems likely that the plant from which collection 42418 was made had come from apomictic ancestry but had by chance received a combination of genes favorable to sexuality. If POWERS' (1945) hypothesis for the evolution of apomixis and polyploidy be applied and we consider each of the three major processes involving the change from diploid sexual plants to polyploid apomictic plants as being differentiated by a single gene, the reversion of an apomictic plant back to a sexual may be explained. Arbitrary factors chosen to explain how this reversion could have occurred are symbolized as follows: *Aa* are alleles differentiating reduction in number of chromosomes as opposed to failure of reduction; *Bb* are alleles differentiating fertilization of egg cell as opposed to failure of fertilization of egg cell; and *Cc* are alleles differentiating

non-development of egg cell into embryo without being fertilized as opposed to development of egg cell into embryo without its being fertilized.

The genotype of the diploid 36± chromosome sexual plant from which the polyploid series may have developed would be *AA BB CC*. The genotype of the triploid 54± chromosome apomict with the greatest possible number of recessive genes would be *aaa bbb ccc*. These recessive genes could be carried along to some extent in the double recessive condition and to a greater extent in heterozygous populations. Heterozygous sexual diploids may carry genes that condition apomixis. By crossing, these genes are brought together. Many hybrids are known to be sterile as to sexual reproduction. Apomixis would have a high survival value and would be favored in natural selection.

Gene combinations producing diploid apomicts would be possible, but apomixis would be more likely among the polyploids with which the process probably evolved. Present apomictic strains would carry genes for apomixis which would accumulate and express themselves in the higher polyploids. Apomixis must have selective value, or more sexuals would appear. Occasional chance combinations of factors for sexuality and accumulations of dominance modifiers would account for the few sexuals which do appear among the polyploids. The sexual plant (42418) whose progeny are described in this article could have arisen by chance combination of several major factors and a favorable combination of modifiers.

SUMMARY

One individual plant selection with high chromosome number among the extensive seed collections made by POWERS and FEDERER in natural populations of guayule and mariola has been shown to be completely sexual. Sexuality has been demonstrated in this strain by data showing variation in percentage resin, percentage rubber, percentage seed germination, size of pollen grains, leaf petiole, and entire plant measurements. Open pollinated and selfed progeny as well as offspring from crosses between members of this strain and three different strains of guayule and one of mariola have been studied. Cytological studies and phenotypic variation observed in progeny demonstrate the sexual nature of the selection. The inheritance of apomixis is discussed.

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GENETICS OF NATURAL POPULATIONS. XII. EXPERIMENTAL REPRODUCTION OF SOME OF THE CHANGES CAUSED BY NATURAL SELECTION IN CERTAIN POPULATIONS OF DROSOPHILA PSEUDOOBSCURA

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THE PROBLEM

RAPID changes in the genetic composition occur in populations of *Drosophila pseudoobscura* which inhabit certain localities on Mount San Jacinto, California (DOBZHANSKY 1943). These changes are cyclic and connected with the succession of the year's seasons. The genetic variable involved is the gene arrangement in the third chromosome. Three gene arrangements are common among the third chromosomes of the San Jacinto populations. One of them, called Standard (abbreviated ST), is most frequent in the populations in winter and in early spring, reaches its lowest frequency in early summer, and increases in frequency during middle and late summer. The second, Chiricahua (abbreviated CH), shows a cycle opposite to that of ST. The third, Arrowhead (AR), tends to follow a path resembling that of CH but with less regularity. Inversion homozygotes and heterozygotes occur in the populations with frequencies which are close to those which are expected if the carriers of the different gene arrangements mate at random.

Analysis of the data has led to the working hypothesis according to which the changes in the relative frequencies of the gene arrangements are induced by natural selection in response to the seasonal alterations in the environment. The gene arrangements may be in themselves adaptively neutral (that is, free from position effects), but they contain different gene complexes which make their carriers adapted to different seasonal environments (DOBZHANSKY 1943). Since the changes in the composition of the populations are considerable and rapid, the chromosomal types concerned must be subject to intense selection pressures. The selective advantages and disadvantages that must be postulated are, indeed, high enough to justify an attempt to detect them in laboratory experiments. The present article reports the results of experiments designed to test the validity of the above hypothesis.

APPARATUS

The problem of creating experimental populations of Drosophila even remotely comparable to the free-living ones is not easy. Attempts to solve it by increasing the size of the container in which the flies are bred are unsatisfactory. No matter how large a container, and how much food it may hold, time comes when the population must be transferred to fresh medium, and this presents as yet insuperable difficulties of the sampling technique. The nearest approach to a successful solution of the problem is that of L'HÉRITIER

* Experimental data by TH. DOBZHANSKY, mathematical analysis by SEWALL WRIGHT.

and TEISSIER (1933) and L'HÉRITIER (1937), who devised population cages in which fresh food is introduced and from which the worked-out food is removed, frequently enough for the size and the age distribution of the population in the cage to remain approximately stationary. We had the privilege also of inspecting a very different model of population cage built by Mrs. G. W. DIEDERICH.

Population cages used in the present experiments (fig. 1) are a modification of the L'HÉRITIER and TEISSIER model. The cage is a wooden box with out-

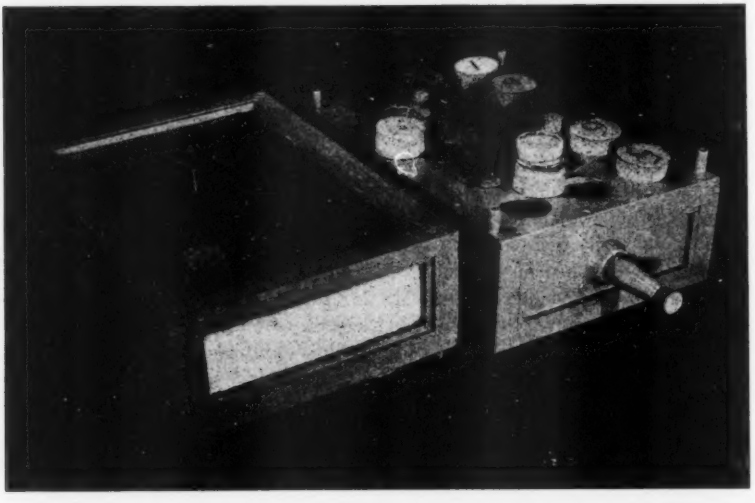

FIGURE 1.—The population cage. Left—view from above; right—view from below.

side dimensions $17 \times 12 \times 5\frac{1}{2}$ inches. The bottom has 15 circular openings $2\frac{1}{4}$ inches in diameter closed by tightly fitting corks. The corks are provided with wire loops that hold in place 2×1 inch Stender jars filled with cornmeal-molasses-agar culture medium. The top of the box and the long sides have glass windows. One of the shorter sides has a window for ventilation covered with wire and gauze nettings to prevent entry and escape of flies. The opposite side has a metallic funnel closed by a cork; this funnel serves as an opening through which flies can be introduced and withdrawn from the cage, and also for cleaning the glass and moistening the food while flies are breeding in the cage.

A known mixture of desired genetic types of flies, together with two or three jars with food, is introduced into the cage at the beginning of the experiment. Jars with fresh food are added, and those with worked-out food removed, at desired intervals. The withdrawal of a cork, removal and replacement of jars with food, and insertion of the cork back into its place can be done with few or no flies escaping. If the number of flies in the cage at the

beginning of an experiment is small, the population increases very rapidly to numbers compatible with the quantity of food in the medium, and thereafter fluctuates within relatively narrow limits. The size of the definitive population at room temperature, and with one jar of fresh food inserted into the cage on alternate days, is of the order of 3000–5000 flies per cage. This population is reached in general within one generation from the introduction of the parent flies into the cage.

The greatest difficulty of experiments with population cages is that any mite infection that may develop in the cage is uncontrollable. As soon as mites appear, the experiment must be terminated and the cage with its contents sterilized by heat. A relatively minor difficulty is that the glass windows of the cage eventually become opaque because of the fly excreta. The glass is cleaned with the aid of a wad of cotton on a wire introduced through the funnel in the side of the cage. The food in the jars inside the cage may become too dry, in which case it is moistened with weak yeast suspension injected through a glass tube.

MATERIAL

All the flies that served as material for the experiments were descendants of individuals collected at Piñon Flats, Mount San Jacinto, California, in the summer of 1942. Chromosomes with Standard, Arrowhead, Chiricahua, Tree-Line, and Santa Cruz gene arrangements are encountered in the populations of that locality, the first three arrangements being much more frequent than the others, particularly than the fifth. Observations extending over four breeding seasons (1939–1942) showed that the relative frequencies of these gene arrangements change from month to month as indicated in table 1 and figure 2 (for further details see DOBZHANSKY 1943).

TABLE 1

Seasonal changes in the percentage frequencies of the different gene arrangements in the third chromosomes of the population of Piñon Flats, California. The symbol "n" in this and all following tables indicates the numbers of the chromosomes examined.

MONTH	ST	AR	CH	OTHERS	n
March	47.2	18.1	28.6	6.0	496
April	46.8	24.9	23.6	4.6	449
May	33.6	29.0	31.3	6.0	642
June	29.2	30.6	35.9	4.3	630
July–August	42.3	26.3	27.3	4.1	388
September	47.3	22.2	26.6	3.9	338
Oct.–Dec.	47.3	26.7	21.2	4.8	330

The frequency of ST is lowest in June, increases during the summer, remains uniformly high during autumn and winter, and decreases in spring. That of CH is highest in June, wanes in summer, is low in winter, and waxes during spring. AR behaves like CH, but the trends are less regular. No significant changes are established for the relatively rare Tree Line and Santa Cruz chromosomes.

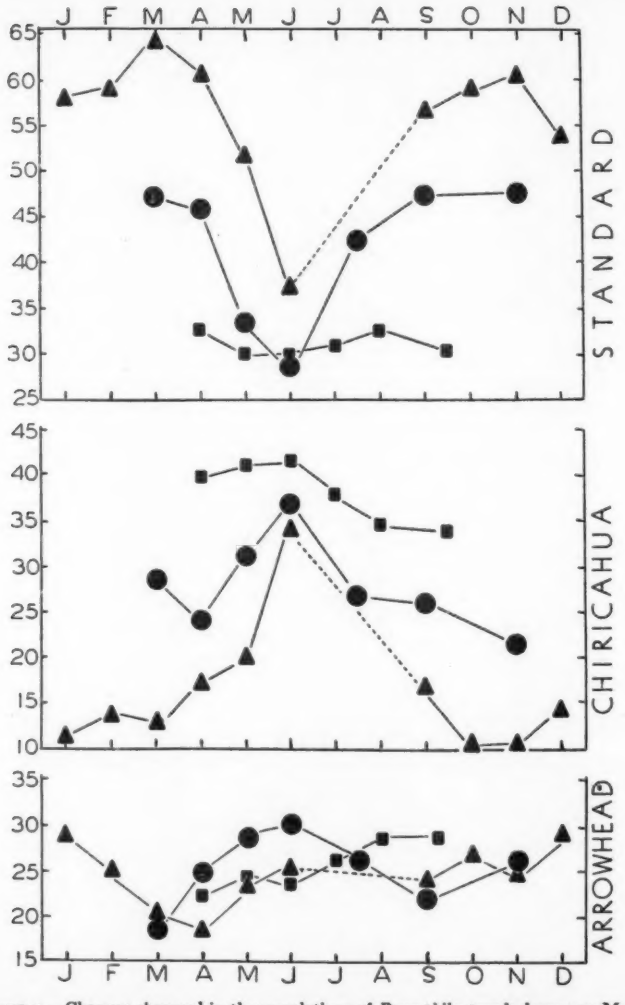

FIGURE 2.—Changes observed in the populations of *Drosophila pseudoobscura* on Mount San Jacinto, California. The figures on the left indicate the percentage frequencies of the three gene arrangements (Standard, Chiricahua, and Arrowhead); the letters at the top and at the bottom stand for the months of the year; triangles—the Andreas Canyon population; circles—the Pinon Flats population; squares—the Keen Camp population. Combined data for four years of observation.

A collection of strains descended from progenitors captured at Piñon Flats has been maintained in the laboratory since 1942; each strain is derived from a single female impregnated by one or more males. Since each female and each male progenitor carried two third chromosomes, which might have had different gene arrangements and gene contents, a strain may harbor four or more kinds of third chromosomes. However, after many generations in the laboratory, some of the ancestral chromosomes may be broken up by crossing over or lost by inbreeding, even though the strains are perpetuated in mass cultures. Theoretically, every strain kept in the laboratory should eventually become homozygous for a single kind of third chromosome. That such an uniformity has not been reached in at least some of the strains is attested by the fact that they continue to carry two or even three chromosomes with different gene arrangements. Retention in laboratory stocks of variability brought in from natural populations is observed even in strains which have have been in captivity for a decade and longer without precautions against inbreeding; some of these stocks still carry third chromosomes with different gene arrangements. A possible reason for this maintenance of variability is that many wild chromosomes carry recessive mutant genes which are deleterious when homozygous; there is a strong selection against homozygosis.

THE INITIAL POPULATION OF AN EXPERIMENTAL CAGE

In the experiments described in this report a fly population with known frequencies of different types of third chromosomes was introduced into a cage, and the incidence of these chromosome types in the progeny of the initial population was observed from time to time. The gene arrangements were determined through examination of the chromosomes in the salivary gland cells of larvae, which were, of course, killed in the process. Nevertheless, given a collection of strains with the desired gene arrangements, populations can be prepared with any initial frequencies of these chromosome types.

For this purpose, two or three dozen pair matings are made from the flies of the strains available. Eight fully grown larvae are taken from the offspring of each pair, their salivary glands stained in acetic orcein, and the gene arrangements in their third chromosomes determined by microscopic examination. With three gene arrangements, ST, AR, and CH, the following twenty-one types of matings are possible (the gene arrangements in the two chromosomes of a zygote are separated by the sign /).

PARENTS	OFFSPRING	PARENTS	OFFSPRING
ST/ST, ST/ST	ST/ST	CH/CH, CH/CH	CH/CH
ST/ST, AR/AR	ST/AR	CH/CH, ST/AR	ST/CH, AR/CH
ST/ST, CH/CH	ST/CH	CH/CH, ST/CH	ST/CH, CH/CH
ST/ST, ST/AR	ST/ST, ST/AR	CH/CH, AR/CH	AR/CH, CH/CH
ST/ST, ST/CH	ST/ST, ST/CH	ST/AR, ST/AR	ST/ST, ST/AR, AR/AR
ST/ST, AR/CH	ST/AR, ST/CH	ST/AR, ST/CH	ST/ST, ST/CH, ST/AR, AR/CH
AR/AR, AR/AR	AR/AR	ST/AR, AR/CH	AR/AR, ST/AR, ST/CH, AR/CH
AR/AR, CH/CH	AR/CH	ST/CH, ST/CH	ST/ST, ST/CH, CH/CH
AR/AR, ST/AR	AR/AR, ST/AR	ST/CH, AR/CH	CH/CH, ST/AR, ST/CH, AR/CH
AR/AR, ST/CH	AR/ST, AR/CH	AR/CH, AR/CH	AR/AR, AR/CH, CH/CH
AR/AR, AR/CH	AR/AR, AR/CH		

The chromosome constitution of the parental pair in each mating thus becomes known through examination of the chromosomes of eight or more of their offspring. The proportions of individuals with different combinations of chromosomes in the whole offspring of the pair is easily deduced. Thus, if one parent is ST/ST and the other ST/AR, half of the offspring are ST/ST and the other half ST/AR; if the parents are ST/AR and ST/AR, the offspring are one-quarter ST/ST, one-half ST/AR, and one-quarter AR/AR. When the adult offspring hatch, the desired numbers of flies are taken from each bottle, the sexes are separated, and the flies are aged for three to four days, then placed into the cage. A simple addition shows the most probable numbers of "chromosomes" with each gene arrangement introduced into the population (each fly, of course, has two "chromosomes"). These numbers in the initial populations in the different experiments are indicated in table 2.

TABLE 2

Conditions in the different experiments.

EXPERIMENT NO.	STARTED	TEMP.	LIGHT	INITIAL NUMBER OF FLIES	NUMBER OF CHROMOSOMES			KINDS OF CHROMOSOMES		
					ST	AR	CH	ST	AR	CH
1	Dec. 8, 1942	Room	Light	480	280	320	360	7	9	8
2	Dec. 8, 1942	Room	Light	480	280	320	360	7	9	8
3	Feb. 1, 1943	16½°	Dark	830	605	495	560	10	6	9
4	Feb. 3, 1943	21°	Dark	620	437	400	403	9	8	9
5	Feb. 3, 1943	25½°	Dark	585	399	372	399	9	6	9
6	Jan. 19, 1944	25½°	Dark	456	253	312	347	6	6	7
7	Jan. 19, 1944	16½°	Dark	503	303	331	372	6	6	7
8	Jan. 22, 1944	25½°	Dark	258	242	—	274	4	—	5
9	Jan. 22, 1944	16½°	Dark	226	207	—	245	4	—	5
10	June 20, 1944	Room	Light	±5000	?	?	?	6	6	7
11	June 20, 1944	Room	Light	±5000	?	?	?	4	—	5
12	June 28, 1944	Room	Light	1027	393	783	878	8	7	9
13	July 18, 1944	Room	Light	1390	813	—	1967	4	—	7
14	July 18, 1944	Room	Light	1002	619	1385	—	5	6	—
15	July 26, 1944	Room	Light	1464	—	930	1998	—	4	7
16	Sept. 30, 1944	16½°	Dark	897	1214	374	206	9	6	5
17	Sept. 30, 1944	16½°	Dark	562	934	—	190	5	—	4
18	Oct. 23, 1944	Room	Light	1356	539	1178	985	9	7	6
19	Nov. 15, 1944	Room	Light	639	489	—	789	5	—	6

Many chromosomes in natural populations of *D. pseudoobscura* carry recessive genes or gene complexes which, when homozygous, modify the viability, fertility, or development rate of the flies (DOBZHANSKY, HOLZ, and SPASSKY 1942). Changes that may be observed in the composition of the population of an experimental cage need not necessarily be ascribed to the properties of the chromosomes with different gene arrangements; such changes may be provoked also by differences in the gene contents of the chromosomes,

which may be independent of the gene arrangement. We are interested, however, in the viability of carriers not of individual third chromosomes but of the ST, AR, and CH chromosomes as groups. Therefore it became necessary to eliminate or minimize the disturbing effects of the gene contents that vary from chromosome to chromosome with the same gene arrangement. To this end, several strains of flies with the same gene arrangement were used; in other words, the ST, AR, and CH chromosomes in the initial population of every experimental cage were derived from several different flies collected on Mount San Jacinto, frequently at different times, weeks or even months apart. The population which develops in a cage contains many individuals homozygous for the ST, AR, and CH gene arrangements. But a majority of these structural homozygotes carried two *different* chromosomes with somewhat different gene contents—that is, they were genic heterozygotes. Table 2 shows the numbers of "kinds of chromosomes" with each gene arrangement in the several experiments. In computing these numbers, it was assumed that every strain contains one and only one kind of third chromosome with each gene arrangement found in that strain. Since more than a single kind were doubtless present in some of the strains, the numbers shown in table 2 are minimum values.

SAMPLING

In order to determine the frequencies of chromosomes with each gene arrangement at different times in the experimental populations, samples were taken at approximately monthly intervals in each cage. The surface of the food in the Stender jars inserted into a cage becomes covered with fly eggs in about 24 hours. A bit of this food was cut out and placed in a regular culture bottle. Extra yeast was added when middle-sized larvae appeared. When the larvae were fully grown, their salivary glands were stained in acetic orcein; examination of the configuration of the third chromosome in one such larva permits determination of the gene arrangements in two third chromosomes, the maternal and the paternal ones. The examination of sufficient larvae should provide a fair estimate of the composition of the population in the cage.

A source of error, however, must be considered. The eggs deposited in a jar come from some but not from all the flies in the cage. To make the samples as representative as possible, the monthly samples of 300 chromosomes were sub-divided into six (seven in the early experiments) subsamples. That is, a chip of food with eggs was taken on each of six successive days, and the chromosomes of 25 larvae from each subsample were examined. This gave six groups of 50 chromosomes each, a total of 300 chromosomes. Comparison of the subsamples constituting a sample should indicate the extent to which the samples are representative of the population of a cage; χ^2's were computed to measure the homogeneity of the subsamples in 38 different samples. Among the 38 χ^2's, 15 had the probability 0.5 or higher of occurring by chance; 12 had probabilities ranging from 0.5 and 0.1; four probabilities from 0.1 to 0.05; three probabilities between 0.05 and 0.02; two between 0.02 and 0.01; and two probabilities just below the 0.01 level. The variability of the sub-

samples constituting a sample, therefore, was in excess of what might be expected owing to chance alone, but only slightly so. A sample may be considered a fair measure of the status of the population of a cage at the time when it is taken.

It follows from the above that the process of taking the monthly sample in an experimental cage occupies nearly a week. In tables 3 to 10 the dates of the samples are given in terms of ten-day periods; E, M, and L before the name of the month indicate the first, second, and third ten-day period, respectively. The entries in tables 3 to 10 labelled "Initial" indicate the composition of the initial population of the experimental cage, as deduced from the chromosomal constitution of the parent flies (see above). The lack of the "Initial" entries in Experiments 10 and 11 (tables 6, 10) is due to the fact that these experiments represent continuation at room temperature of Experiments 7 and 9 (tables 5, 8) carried at a lower temperature; the initial populations in Experiments 10 and 11, therefore, are the same as the final populations in the cages in Experiments 7 and 9, respectively, and their compositions are indicated by the "Control" entries.

In most experiments, a "Control" sample was taken about a week after the introduction of the initial fly population into the cage. The eggs collected at that time are bound to come from the initial population, since the F_1 generation begins to hatch in no less than two weeks from the start of the experimental cage. The incidences of the several gene arrangements in the Control sample therefore should be the same as in the initial population. Yet, more or less significant differences were observed in the second, fifth, and ninth experiments. It is possible that these differences were due merely to sampling errors. On the other hand, it should be kept in mind that the composition of the initial population is inferred from that of its parents, while the Control sample describes the genetic constitution of the eggs being deposited in the cage about a week after the beginning of the experiment. In other words, a whole generation elapses between the points to which these two sets of data are relevant. If the carriers of some gene arrangements are more and of others less favored in certain environments, differences between the Initial and Control populations may be real. It may be noted in this connection that the cultures from which the flies of the initial populations came in all experiments developed at room temperature. Since at that temperature ST chromosomes are more viable than CH chromosomes (see below), control samples are liable to show higher frequencies of ST and lower ones of CH than the corresponding initial populations. This is what is actually found wherever the two are appreciably different.

In natural populations the carriers of the different gene arrangements interbreed at random. The frequencies of inversion homozygotes and heterozygotes are in accord with expectations based on the Hardy-Weinberg theorem (DOBZHANSKY and EPLING 1944, and other work). The same holds for our samples of the populations of the experimental cages. Only the gametic frequencies of the gene arrangements are reported in tables 3 to 10, the bulkier zygotic data being kept on file.

LENGTH OF THE LIFE CYCLE

Almost from the time the initial population is introduced into an experimental cage, the number of larvae in the jars is much greater than can mature on the food available. Under these rigorous conditions the duration of the development from egg to adult is greater in experimental cages than it is in environments more nearly approaching optimum. The mass hatching of adults from pupae takes place 24 to 26 days after the insertion of the jar with food into the experimental cage at 25½°C, in about 28 to 31 days at 21°C, and in about 35 to 37 days at 16½°C. Some flies hatch both earlier and much later than the times indicated, but it is the date of mass hatching that is most important in this investigation, and the above figures represent the best estimates based on visual observation of jars in different experimental cages. The longevity of the flies in the cages is probably smaller than it would be under optimal conditions, and a minority of members of the initial population live long enough to meet the F_1 individuals appearing in the cage.

TABLE 3

Percentage frequencies of the different gene arrangements. First experiment—abundant food, second experiment—food scarce.

TIME	EXPERIMENT 1				EXPERIMENT 2			
	ST	AR	CH	n	ST	AR	CH	n
Initial	29.2	33.3	37.5		29.2	33.3	37.5	
Control	27.1	35.0	38.0	266	34.5	39.9	25.6	258
E January 1943	47.3	30.8	21.9	370	44.5	37.2	18.3	328
E February 1943	42.4	38.6	19.0	342	46.0	35.3	18.6	354
M April 1943	54.3	27.1	18.6	376	50.5	29.4	20.1	364
M May 1943	54.2	19.2	26.5	426	55.8	28.9	15.3	398
M June 1943	52.9	27.1	20.1	384	53.7	33.0	13.3	300

RESULTS

Among the 19 experiments performed, no two were sufficiently alike to be properly considered replications. Some of the variables are given in table 2.

The first and second experiments (table 3) were designed to test the possible influence of the food regime. In the first experiment, two jars with fresh food were given every three days. Since an experimental cage has 15 food jars (see above), each jar in the first experiment remained in the cage for 22 to 23 days and when removed contained many unhatched pupae. This puts a prize on rapid development, because all slowly developing individuals are eliminated. In the second experiment, one jar with fresh food was introduced every five days. Therefore, each jar remained in the cage for 75 days, and when finally removed, it contained no live larvae or pupae and the food was entirely consumed. The two cages stood side by side in a room which was kept very warm —25–27°C during the day, from a fraction to three degrees lower during the night. The initial populations of the cages, started in early December of 1942,

contained approximately equal proportions of ST, AR, and CH chromosomes. The frequencies of ST rose and those of CH dropped with time in both cages. In about two months from the start, in early February of 1943, the frequency of ST reached 42 to 46 percent and CH declined to about 19 percent; AR remained at about the initial level (table 3). In other words, the frequency of CH was reduced in two to three generations to about half of its former value. From early February to the middle of June, when both experiments were discontinued because of mite infection, no sharp changes occurred in the populations; the samples show a further increase of the frequency of ST and a slight decline of AR in Experiment 1, and an increase of ST and a decline of CH in Experiment 2. No consistent differences in the trends which could be ascribed to the different food regimes became apparent in the two experiments. As shown below, the waxing of the frequencies of ST and the waning of CH occurred in all experiments conducted at intermediate and high temperatures.

TABLE 4

Percentage frequencies of the different gene arrangements. Third experiment—16½°C, fourth experiment—21°C, fifth experiment—25½°C.

TIME	EXPERIMENT 3				EXPERIMENT 4				EXPERIMENT 5			
	ST	AR	CH	n	ST	AR	CH	n	ST	AR	CH	n
Initial	36.5	29.8	33.7		35.2	32.3	32.5		34.1	31.8	34.1	
Control	34.7	31.2	34.1	320	41.4	31.4	27.2	360	44.2	37.1	18.7	310
M April 1943	50.0	22.3	27.7	300	45.0	32.0	23.0	300	54.0	31.7	14.3	300
M May 1943	—	—	—		46.0	29.3	24.7	300	55.7	33.7	10.7	300
M June 1943	36.0	32.0	32.0	300	45.0	29.7	25.3	300	56.1	30.1	13.8	362
M July 1943	—	—	—		47.6	39.6	12.8	250	57.3	30.7	12.0	300

Experiments 3, 4, and 5 were started early in February of 1943 in order to test the influence of temperature (table 4). The cages were placed in incubators at 21° and 25½°C and in a cold room at 16½°C. One jar of fresh food was given every four days in Experiment 3, every three days in Experiment 4, and on alternate days in Experiment 5; consequently, the jars remained in the cages 60, 45, and 30 days respectively, which at the low, intermediate and the high temperatures suffices for a majority of the surviving larvae and pupae to become transformed into imagoes. The initial populations contained about equal proportions of ST, AR, and CH chromosomes. Rapid increases in the frequencies of ST and decreases of CH, with AR remaining at about the same level, were observed in the fourth and fifth experiments (table 4). The changes seemed to be more rapid in the experiment (the fifth) conducted at the higher than in that (the fourth) conducted at the intermediate temperature, but the difference between the control sample and the initial population in Experiment 5 makes this uncertain. The result of Experiment 3 is somewhat ambiguous. Here the control sample coincided very well with the presumed composition of the initial population; a sample taken two months after the start, in the

middle of April of 1943, showed an ostensible rise of ST and a drop of CH; no sample was taken in May, but the mid-June sample (when the experiment was discontinued on account of mite infection) agreed almost exactly with the control and with the initial population (table 4).

TABLE 5

Percentage frequencies of the different gene arrangements. Sixth and eighth experiments—25½°C seventh and ninth experiments—16½°C.

TIME	ST	AR	CH	n	ST	CH	n
		EXPERIMENT 6				EXPERIMENT 8	
Initial	27.7	34.2	38.1		46.9	53.1	
Control	29.0	28.3	42.7	300	52.0	48.0	300
L February 1944	34.0	31.0	35.0	300	60.3	39.7	300
M March 1944	45.7	26.0	28.3	300	63.3	36.7	300
M April 1944	44.3	21.0	34.7	300	69.3	30.7	250
		EXPERIMENT 7				EXPERIMENT 9	
Initial	30.1	32.9	37.0		45.8	54.2	
Control	34.7	23.0	42.3	300	59.0	41.0	300
L March 1944	35.7	26.3	38.0	300	48.3	51.7	300
L April 1944	33.8	25.2	41.0	290	51.0	49.0	300
E June 1944	35.7	24.7	39.7	300	46.3	53.7	300

To establish beyond doubt the influence of the temperature factor indicated by the preceding experiments, four cages were started in January of 1944 (Experiments 6, 7, 8, and 9, table 5). Cages Nos. 6 and 8 were placed in an incubator at 25½°C, and Nos. 7 and 9 in a cold room at 16½°C. Since the foregoing experiments indicated that the frequencies of ST and CH are more subject to change than those of AR, cages Nos. 8 and 9 were populated by flies with ST and CH chromosomes only. All three gene arrangements were introduced into cages Nos. 6 and 7. Slightly more CH than ST chromosomes were put into the initial mixtures in all four experiments under consideration. The frequencies of ST rose rather sharply in the two experiments at the higher temperature, even though these experiments lasted only three months, till mid-April of 1944. The frequency of CH dropped in the eighth experiment, while in the sixth both AR and CH appear to have shared in the decline (table 5). In the eighth experiment the frequency of CH chromosomes was equal to or greater than that of ST chromosomes in January, but in April ST chromosomes were twice as frequent as CH. Contrasting with the results of the experiments at the high temperature, no appreciable and consistent changes in the frequencies of the gene arrangements occurred in the seventh and ninth experiments which lasted for five months (January–June) but which were carried on in the cold room (table 5). It may be noted that the initial populations of cages Nos. 6 to 9 consisted of sibs. The difference in the results can be ascribed only to temperature.

In June of 1944, experimental cages Nos. 7 and 9 were taken out of the cold

TABLE 6

Percentage frequencies of the different gene arrangements. Tenth to 12th experiments.

TIME	EXPERIMENT 10				EXPERIMENT 11			EXPERIMENT 12			
	ST	AR	CH	n	ST	CH	n	ST	AR	CH	n
Initial	—	—	—		—	—		19.1	38.1	42.7	
Control	35.7	24.7	39.7	300	46.3	53.7	300	22.0	38.3	39.7	300
L July 1944	38.7	25.7	35.7	300	61.7	38.3	300	28.0	40.6	31.4	300
M August 1944	52.3	19.7	28.0	300	66.3	33.7	300	39.7	35.3	25.0	300
M September 1944	48.3	22.3	29.3	300	63.7	36.3	300	50.7	30.3	19.0	300

room and placed first in a room with an open window, where they experienced the vagaries of the summer temperatures, and in late August they were transferred to an incubator at 25°C. The numbers of these cages were changed to 10 and 11, respectively. When removed from the cold room, the cages contained numerically fully developed populations. The last (June) samples in cages Nos. 7 and 9 (see table 5) may therefore be regarded as control samples in cages Nos. 10 and 11 (table 6). The temperature change was soon reflected in the incidence of the gene arrangements. Already the July samples indicated an upward trend for ST and a downward one for CH, and by mid-August the changes became quite significant. The experiments had to be discontinued in September on account of an infection; the last samples did not differ from the August ones.

TABLE 7

Percentage frequencies of the different gene arrangements. Thirteenth to 15th experiments.

TIME	EXPERIMENT 13			EXPERIMENT 14			EXPERIMENT 15		
	ST	CH	n	ST	AR	n	AR	CH	n
Initial	29.2	70.8		30.9	69.1		31.8	68.2	
L August 1944	54.7	45.3	300	48.3	51.7	300	43.0	57.0	300
M September 1944	58.3	41.7	300	55.3	44.7	300	42.0	58.0	300

Late in June of 1944, a mixture of flies in which CH and AR chromosomes were each about twice as frequent as ST ones was introduced into cage No. 12 (table 6). The environmental conditions in the 12th experiment were the same as in the 10th and the 11th (see above). In a month, in late July, the frequency of ST rose from 19 percent (or from 22 percent if the control sample is taken as the starting point) to 28 percent, and the frequency of CH fell from 43 percent (or 40 percent) to 31 percent. By mid-September ST rose to 51 percent and CH fell to only 19 percent; the frequency of AR dwindled relatively slightly. Thus in only three months—that is, in three to four generations—the frequency of ST was more than doubled, and that of CH reduced to less than half of the initial value.

It is clear that under the conditions of these experiments ST chromosomes displace CH chromosomes at high but not at low temperatures. The behavior of AR chromosomes is erratic: they either hold their own or else lose in competition with ST. To test this point further, in July of 1944 experiments 13, 14, and 15 were designed in which only two gene arrangements were introduced into each cage: ST and CH in the 13th, ST and AR in the 14th, and AR and CH in the 15th (table 7). The cages were kept first in a laboratory room with closed windows, where the populations were injured by the summer heat, then in a well ventilated laboratory room, and finally in an incubator at 25°C. By mid-September all cages developed a mite infection and had to be destroyed; thus, the experiments lasted less than two months. The results, nevertheless, are fairly clear. In the 13th experiment the frequency of ST doubled while that of CH fell from 71 percent to 42 percent. This is consistent with the results of the eighth and the 11th experiments (tables 5 and 6), in which the ST and CH gene arrangements were competing at a high temperature; at the low temperature (experiment 9, table 5, and experiment 19, table 9) the relative frequencies of ST and CH remained constant. In the 14th experiment (table 7) ST increased in frequency from 31 percent to 55 percent, while AR correspondingly dwindled from 69 percent to 45 percent. It follows that at high temperatures chromosomes with ST gene arrangement displace those with AR, provided at least, that CH chromosomes, which are still weaker competitors than AR, are absent. A comparison of the results of the 13th and the 14th experiments suggests, if the data are taken at their face value, that the displacement of CH by ST is more rapid than the displacement of AR by ST; the difference, however, is not statistically significant. The result of the 15th experiment (table 7) is not satisfactory: the August sample indicates that AR chromosomes displace CH in the absence of ST, which is consistent with the expectation, but the September sample failed to show a further drop in the frequency of CH.

The results of the experiments so far described are of two kinds. Namely, in the experiments conducted at the higher temperatures the frequencies of ST chromosomes increase and those of CH decrease, while at 16½°C the proportions of ST, AR, and CH gene arrangements show no perceptible alterations. It is known, however, that in the population of the Piñon Flats locality from which the ancestors of the experimental flies came, a third kind of behavior of the gene arrangements is observed every spring season: the frequency of ST drops and that of CH increases. An unsuccessful attempt to reproduce experimentally this last type of change has been made. It is very probable that neither homozygotes for ST (ST/ST) nor for CH (CH/CH), but the heterozygotes ST/CH are the most favored genetic constitution. If so, the changes in the populations will proceed toward equilibrium values at which the relative proportions of the three genotypes will give the maximal degree of fitness of the population. The equilibrium values may, of course, be different at different temperatures. Now, in experiments 1 to 17 the initial populations contained either equal proportions of ST and CH, or else ST was less frequent than CH. It is possible therefore that at the low temperature these initial

populations happened to contain just about the equilibrium proportions of the gene arrangements. If so, no striking changes in the composition of the populations could be expected in these experiments. Accordingly, experiments 16 and 17 were started at 16½°C with initial populations containing considerably more ST than CH chromosomes (table 8). The populations were kept in the cold room from September 30, 1944, till February 12, 1945, without consistent changes in the frequencies of the gene arrangements being observed. This suggests that the adaptive values of the homo- and heterozygotes for the three gene arrangements are very nearly alike in experimental cages kept at 16½°C.

TABLE 8

Percentage frequencies of the different gene arrangements. Sixteenth and 17th experiments. Temperature 16½°C.

TIME	EXPERIMENT 16				EXPERIMENT 17		
	ST	AR	CH	n	ST	CH	n
Initial	67.7	20.9	11.5		83.1	16.9	
L October 1944	76.3	9.3	14.3	300	86.7	13.3	300
M November 1944	63.0	19.7	17.3	300	87.7	12.3	300
L December 1944	66.0	16.3	17.6	300	89.7	10.3	300
E February 1945	61.0	24.3	14.7	300	86.3	13.7	300

STATISTICAL EVIDENCE OF SELECTION

A statistical study of the changes in chromosome frequency has been based on a grouping of the experiments according to the chromosomes present and the temperature. The largest group involved all three chromosomes at 25° (experiments 1, 2, 5, 6, 10, 12, and 18). These provide data on the changes in 30 intervals, disregarding the estimates of the initial compositions of the boxes. Experiments 3, 7, and 16 (eight intervals) involve all three chromosomes at 16.5°. Experiments 8, 11, 13, and 19 (13 intervals) involve Standard and Chiricahua at 25°, and experiments 9 and 17 (6 intervals) involve the same chromosomes at 16.5°. There remain three isolated experiments, No. 14 involving Standard and Arrowhead at 25°, No. 15 involving Arrowhead and Chiricahua at 25°, and No. 4 involving all three chromosomes at 21°. These three experiments will not be considered here beyond calling attention to the fact that the changes are in the directions expected from the other experiments.

In making a statistical analysis, it is necessary to reduce the observed changes in chromosome frequency to rates per generation. These were estimated on the assumption of an interval of 3.5 weeks between generations in the experiments at 25° and an interval of 5.2 weeks in those at 16.5°.

The first question is the statistical significance of any changes in composition that may indicate selection. Both the average rate of change per generation and the regression of rate of change on chromosome frequency must be considered. A significant average rate of change obviously indicates a selective

process, but even if the average does not differ significantly from zero, a significant regression indicates a selective process which changes in amount or even in direction as chromosome frequency changes.

The principal results in the four groups of experiments referred to above are shown in table 10. Here n is the number of intervals, $\bar{q}$ is the mean chromosome

TABLE 9

Percentage frequencies of the different gene arrangements. Eighteenth and 19th experiments. Room temperature.

TIME	EXPERIMENT 18				EXPERIMENT 19		
	ST	AR	CH	n	ST	CH	n
Initial	19.9	43.6	36.5		38.3	61.7	
M November 1944	33.3	27.3	39.3	300	—	—	
M December 1944	37.7	28.7	33.7	300	53.0	47.0	300
M January 1945	39.3	30.0	30.7	300	63.3	36.7	300
L February 1945	44.3	30.0	25.7	300	60.3	39.7	300
L March 1945	42.0	39.0	19.0	300	65.3	34.7	300
L April 1945	46.7	30.3	23.0	300	65.3	34.7	300
E June 1945	56.4	27.3	16.3	282	70.4	29.6	250
L July 1945	50.3	31.7	18.0	300	72.0	28.0	300

frequency; $\bar{\Delta}q$ is the mean rate of change of chromosome frequency per generation; $b_{\Delta q \cdot q}$ is the regression of rate of change on chromosome frequency, and $\sigma^2_{\Delta q \cdot q}$ is the variance of Δq estimated for constant q. The significance of $\bar{\Delta}q$ and $b_{\Delta q \cdot q}$ are determined from their ratios (t) to their standard errors ($SE_{\bar{\Delta}\bar{q}}$ and SE_b, respectively), using Students' probabilities for small numbers.

TABLE 10

Statistical analysis of the experimental data (further explanation in text)

	TWO TYPES AT 25°	THREE TYPES AT 25°			TWO TYPES AT 16.5°	THREE TYPES AT 16.5°		
	STANDARD (VS. CHIRICAHUA)	STANDARD	ARROWHEAD	CHIRICAHUA	STANDARD (VS. CHIRICAHUA)	STANDARD	ARROWHEAD	CHIRICAHUA
n	13	30	30	30	6	8	8	8
$\bar{q}$	.5492	.4201	.3182	.2617	.7040	.4927	.2169	.2904
$\bar{\Delta q}$	+.0472	+.0391	−.0122	−.0168	−.0125	−.0156	+.0167	−.0011
$SE_{\bar{\Delta q}}$	.0061	.0076	.0068	.0072	.0162	.0190	.0110	.0102
t	7.77	5.17	1.80	3.74	.77	.82	1.53	.11
Prob.	<.001	<.001	.05–.10	<.001	.40–.50	.40–.50	.10–.20	>.90
$b_{\Delta q \cdot q}$	−.346	−.387	−.394	−.261	+.036	−.237	−.614	−.055
SE_b	.057	.074	.116	.079	.092	.119	.176	.095
t	6.13	5.21	3.40	3.31	.39	1.99	3.48	.58
Prob.	<.001	<.001	<.01	<.01	.70–.80	.05–.10	.01–.02	.50–.60
$\sigma^2_{\Delta q \cdot q}$	.00048	.00172	.00139	.00154	.00163	.00290	.00096	.00083
$\bar{q}(1-\bar{q})/306$	.00079	.00078	.00071	.00062	.00059	.00075	.00055	.00065
F	.61	2.21	1.95	2.51	2.77	3.88	1.74	1.28
Prob.	.05–.20	<.001	<.01	<.001	.01–.05	<.001	.05–.20	>.20

The variance of Δq for given q is compared with that expected from accidents of sampling in a sample of 300 by Fisher's test. The following formulae were used:

$$\bar{q} = \sum q/n$$
$$\overline{\Delta q} = \sum \Delta q/n$$
$$b_{\Delta q \cdot q} = (\sum q\Delta q - \overline{\Delta q}\sum q)/(\sum q^2 - \bar{q}\sum q)$$
$$\sigma^2_{\Delta q \cdot q} = [(\sum \Delta^2 q - \overline{\Delta q}\sum \Delta q) - b^2_{\Delta q \cdot q}(\sum q^2 - \bar{q}\sum q)]/(n-2)$$
$$SE^2_{\overline{\Delta q}} = \sigma^2_{\Delta q \cdot q}/n$$
$$SE^2_b = \sigma^2_{\Delta q \cdot q}/(\sum q^2 - \bar{q}\sum q).$$

From inspection of table 10 it may be seen that there were highly significant changes in chromosome frequency in the experiments at 25° in which all three chromosomes were involved and also in those involving only Standard and Chiricahua, in spite of the small number of cases in the latter. In both sets, Standard tended to increase and Chiricahua to decrease within the range of values of chromosome frequencies in the experiments. There were, on the other hand, no significant changes in chromosome frequency in the experiments at 16.5°. This does not imply that there were no changes but merely that any changes were too small to be detected with confidence in the small number of intervals in these sets of experiments.

The regression of rate of change on chromosome frequency was highly significant for all chromosomes in the experiments at 25°. In all cases, the slope is negative and such as to indicate a reversal in the direction of change at some point. In other words, there is a tendency for any chromosome to increase in frequency when rare and decrease when sufficiently common. In the experiments at 16.5° there is one apparently significant result. The rate of change of Arrowhead falls off very rapidly as its frequency increases (probability between .01 and .02). There is a suggestion of a similar trend in the case of Standard (probability between .05 and .10) but not in the case of Chiricahua.

The estimated variance of Δq for given q is actually less, though not significantly, than expected by chance in a sample of 300 in the experiments involving only Standard and Chiricahua at 25°. On the other hand, it is consistently and highly significantly greater for the three chromosomes in the experiments involving all three at 25°. The excess could be accounted for by limitation of the number of parents but could also be due to unknown factors that affected the conditions of selection differently in the various experiments and intervals. If due solely to limitations in the effective number of parents a rough estimate of this effective number (N) may be made by equating the excess $[\sigma^2_{\Delta q \cdot q} - \overline{q(1-q)}/300]$ to $\overline{q(1-q)}/2N$, the variance expected from this cause. The estimate came out 124 from the data on Standard, 157 from that on Arrowhead, and 113 from that on Chiricahua, with an average of 131. It is probable, however, that other factors (density, condition of food, etc.) are the ones that are important.

There is a highly significant excess variance of Δq for given q at 16.5° in

the case of Standard, not borne out by corresponding excesses in the cases of Arrowhead and Chiricahua where all three are involved. There is also excess variance at this temperature where only Standard and Chiricahua are involved. There can be little doubt that in most cases the rate of change of chromosome frequency, at a given frequency, varies more than accounted for by mere sampling errors.

ESTIMATES OF SELECTIVE VALUES

It is of interest next to attempt to determine the nature of the selective process and the values of the selection coefficients. Unfortunately the data at hand do not yield a unique interpretation.

The simplest assumptions are that the relative selective values of the genotypes are the same at all chromosome frequencies and that they are the same for males and females. We shall consider first the sets of experiments involving only Standard and Chiricahua at 25°. Selection must favor the heterozygotes over both homozygotes, under the above hypotheses, if there is a point of equilibrium (*cf.* FISHER 1922). Let s be the selective disadvantage of homozygous Standard and t that of homozygous Chiricahua, relative to the heterozygotes.

Genotype	*Frequency* (f)	*Selective Value* (W)	
ST/ST	q^2	$1 - s$	
ST/CH	$2q(1 - q)$	1	(1)
CH/CH	$(1 - q)^2$	$1 - t$	

It can easily be found that Δq is related to q by the following formula (*cf.* WRIGHT 1931)

$$\Delta q = q(1 - sq - \overline{W})/\overline{W}, \qquad \overline{W} = 1 - sq^2 - t(1 - q)^2 \tag{2}$$

$$\Delta q = -q(1 - q)[sq - t(1 - q)]/\overline{W} \tag{3}$$

$$s = \frac{1}{q}\left[1 - \left(1 + \frac{\Delta q}{q}\right)\overline{W}\right] \quad \text{from (2)} \tag{4}$$

$$t = \frac{1}{(1 - q)}\left[1 - \left(1 - \frac{\Delta q}{(1 - q)}\right)\overline{W}\right]. \tag{5}$$

The equilibrium point, $\hat{q}$, is that at which $\Delta q = 0$, with both chromosomes present.

$$\hat{q} = t/(s + t) \quad \text{from (3)}. \tag{6}$$

In this set of experiments, observations were made at values of q ranging between .292 and .663. The observed values of Δq do not diverge conspicuously from the linear regression line, $\Delta q = .2375 - .3465q$. This indicates equilibrium at about $\hat{q} = .685 (= .2375/.3465)$, slightly above the highest value of q in the data. No great confidence can be placed in this, however, because of the theoretical curvilinear relation of Δq to q. The best estimate that can be made from the linear regression is probably that obtained by equating the

regression coefficient to the slope of the tangent of the theoretical curve at the mean value of q.

$$\frac{d\Delta q}{dq} = \frac{\Delta q}{q} - q\left[\frac{s}{\overline{W}} + \frac{(1 - sq)}{\overline{W}^2}\frac{d\overline{W}}{dq}\right] \quad \text{from (2)}$$

$$= 2\left(1 + \frac{\Delta q}{q}\right)\left(1 - \frac{\Delta q}{1 - q}\right) - 1 - \frac{1}{\overline{W}} \quad \text{using (4) and (5)} \qquad (7)$$

Assuming that $\frac{d\Delta q}{dq}$ at $\bar{q}$ is approximated by $b_{\Delta q \cdot q}$ and substituting $\bar{\Delta q}$ and $\bar{q}$ for Δq and q respectively, $\overline{W}$ at $\bar{q}$ may be estimated from the reciprocal of the following expression.

$$\frac{1}{\overline{W}} = 2\left(1 + \frac{\Delta q}{q}\right)\left(1 - \frac{\Delta q}{1 - q}\right) - 1 - b_{\Delta q \cdot q}. \qquad (8)$$

Estimates of s and t can now be made by the same substitutions with the help of the estimate for $\overline{W}$ at $\bar{q}$. For Standard and Chiricahua at 25°

$$\begin{aligned} \overline{W} \text{ at } \bar{q} &= .7747 \\ s &= .289 \\ t &= .680 \\ \hat{q} &= .702. \end{aligned} \qquad (9)$$

For the best estimates, however, it is necessary to use the method of least squares. As the expression for Δq in terms of q is not linear with respect to the parameters s and t (equation 3), the solution must be determined by iteration. Using trial values of s and t to estimate $\overline{W}$ for each observation (*cf.* (2)), values of s and t in the numerator of (3) can be obtained that minimize the squared deviations of observed and estimated Δq. The estimates of $\overline{W}$ may then be readjusted and the process repeated until the values of s and t calculated for the numerator agree sufficiently with those tried in the denominator. Strictly, the observations should be weighted by the reciprocals of their variances (that is, in proportion to $q(1-q)$), but as these weights would range only from 4.0 to 4.8 in this data, weighting may be ignored without serious error, in order to avoid the considerable complication which it would introduce into the calculations.

Let $y = \Delta q$, $x_1 = q^2(1-q)/\overline{W}$ and $x_2 = q(1-q)^2/\overline{W}$.

The deviations (δ) of the 13 observations from the theoretical values can be written as follows by substitutions in (3).

$$\delta = y + x_1 s - x_2 t. \qquad (10)$$

Minimizing $\sum \delta^2$ by putting $\frac{\partial \sum \delta^2}{\partial s}$ and $\frac{\partial \sum \delta^2}{\partial t}$ equal to zero gives the normal equations:

$$\begin{aligned} (\sum x_1^2)s - (\sum x_1 x_2)t &= -\sum x_1 y \\ -(\sum x_1 x_2)s + (\sum x_2^2)t &= \sum x_2 y. \end{aligned} \qquad (11)$$

The results of successive trials, starting from the estimates of s and t, arrived at from the regression coefficient, were as follows in terms of the values tried in the denominator and calculated (calc.) for the numerator of (3).

	1ST TRIAL		2ND TRIAL		3RD TRIAL		FINAL
	TRIED	CALC.	TRIED	CALC.	TRIED	CALC.	ESTIMATES
s	.289	.3083	.2987	.3047	.3031	.3038	.304
t	.680	.7037	.6919	.6967	.6949	.6948	.695

The equilibrium point from the final estimates is $\hat{q}=.696$.

The values, $s=.30$, $t=.70$, $\hat{q}=.70$, are as accurate as the data warrant. The solid line in figure 3 shows the theoretical curve for Δq based on these values in comparison with the 13 observations.

This theory obviously fits the data as well as can be expected, but it involves the assumption that selection acts in the same way on males and females. This is not necessarily the case. Selection may occur in such factors as mating activity or fecundity rather than in mortality. It is not likely that there would be the same differentials between genotypes in the males and females in mating and fecundity. It is thus necessary to consider the effect of differences in the selective values of the sexes. Fortunately the results differ little from those obtained by assigning the average for each genotype to both sexes, as noted by WRIGHT (1942).

Let $q_f(=q+\delta q)$ be the frequency of ST in eggs and $q_m(=q-\delta q)$ be that in sperms, and let $W_{11}+\delta W_{11}$ be the selective value of genotype ST/ST in females and $W_{11}-\delta W_{11}$ be that in males, and let similar symbols be used for other selective values. Thus q is the average frequency of ST and the W's are average selective values referred to above.

Selective values

	Frequency (f)	*Females* (W_f)	*Males* (W_m)	*Average*	
ST/ST	$q^2-\delta^2 q$	$W_{11}+\delta W_{11}$	$W_{11}-\delta W_{11}$	W_{11}	
ST/CH	$2q(1-q)+2\delta^2 q$	$W_{12}+\delta W_{12}$	$W_{12}-\delta W_{12}$	W_{12}	(13)
CH/CH	$(1-q)^2-\delta^2 q$	$W_{22}+\delta W_{22}$ z	$W_{22}-\delta W_{22}$	W_{22}	

The frequencies of Standard in eggs ($_1q_f$) and sperms ($_1q_m$) that function in producing the next generation are as follows, using $\overline{W}_f$ for $\sum W_f f$ and $\overline{W}_m$ for $\sum W_m f$.

$$\begin{aligned} {}_1q_f &= \{[q^2-\delta^2 q][W_{11}+\delta W_{11}]+[q(1-q)+\delta^2 q][W_{12}+\delta W_{12}]\}/\overline{W}_f \\ {}_1q_m &= \{[q^2-\delta^2 q][W_{11}-\delta W_{11}]+[q(1-q)+\delta^2 q][W_{12}-\delta W_{12}]\}/\overline{W}_m. \end{aligned} \quad (14)$$

Let $_1q=(1/2)(_1q_f+{}_1q_m)$ be the average frequency of Standard after a generation and $_1\delta q=(1/2)(_1q_f-{}_1q_m)$ be the deviation in females from this average, and let $\overline{W}_f=\overline{W}+\delta\overline{W}$ and $\overline{W}_m=\overline{W}-\delta\overline{W}$.

$$\begin{aligned} {}_1q = \{&[\overline{W}W_{11}-\delta\overline{W}\delta W_{11}][q^2-\delta^2 q] \\ &+[\overline{W}W_{12}-\delta\overline{W}\delta W_{12}][q(1-q)+\delta^2 q]\}/[\overline{W}^2-(\delta\overline{W})^2] \end{aligned} \quad (15)$$

$$\begin{aligned} {}_1\delta q = \{&[\overline{W}\delta W_{11}-W_{11}\delta\overline{W}][q^2-\delta^2 q] \\ &+[\overline{W}\delta W_{12}-W_{12}\delta W_{12}][q(1-q)+\delta^2 q]\}/[\overline{W}^2-(\delta\overline{W})^2]. \end{aligned} \quad (16)$$

It may be noted that the above value of $_1q$ differs from the expression $[W_{11}q^2+W_{12}q(1-q)]/\overline{W}$, expected if selection acts in the same way on males and females, only by terms that are of the second degree with respect to sex differences.

On starting from any initial values of q and δq, the values in subsequent generations can be found by repeated application of (15) and (16).

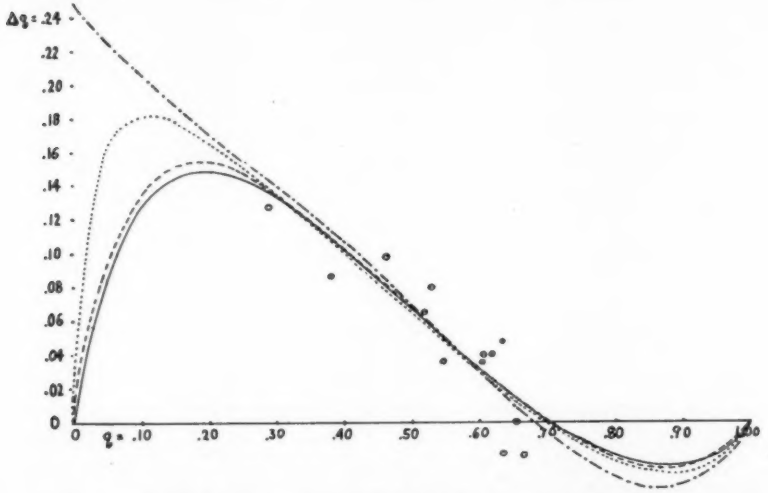

FIGURE 3.—The circles show the amounts of change per generation (Δq) in the frequency (q) of Standard chromosomes in the experiments which involved only Standard and Chiricahua at 25°. The solid line represents the theoretical relation of Δq to q that fits best under the hypothesis that the heterozygotes have a constant selective advantage over both homozygotes and that the sexes are alike in this respect. The broken line represents the same hypothesis except that the homozygotes of one sex are supposed to have considerably more selective disadvantage in relation to the homozygotes than do those of the other sex. The line in dot and dash is the case in which the relative selective disadvantage of the homozygotes in one sex is as extreme as possible. The dotted line represents the hypothesis that the heterozygotes are intermediate between the homozygotes but that each homozygote is favored when rare and opposed when sufficiently common. All hypotheses fit about equally well in the region covered by the experiments.

The effects of considerable differences between the sexes with respect to selection were tested by taking the same average values of W_{11}, W_{12}, and W_{22} arrived at above but assuming δW_{11} and δW_{22} both to be .10.

Genotype	*Selective Vales*			
	W_f	W_m	W	
ST/ST	.80	.60	.70	(17)
ST/CH	1.00	1.00	1.00	
CH/CH	.40	.20	.30	

The calculations were started from q=.01, δq=0 and q=.99, δq=0.

q	δq	Δq	q	δq	Δq	q	δq	Δq
.01	0	+.0252	.99	0	−.0043	.8264	+.0142	−.0249
.0352	−.0110	+.0725	.9857	+.0020	−.0061	.8015	+.0144	−.0221
.1077	−.0285	+.1375	.9796	+.0028	−.0084	.7794	+.0143	−.0187
.2452	−.0425	+.1475	.9712	+.0038	−.0113	.7607	+.0138	−.0152
.3927	−.0321	+.1062	.9599	+.0052	−.0147	.7455	+.0133	−.0121
.4989	−.0153	+.0673	.9452	+.0068	−.0185	.7334		−.010
.5662	−.0046	+.0425	.9267	+.0086	−.0222			
.6087	+.0014	+.0275	.9045	+.0104	−.0250			
.6362	+.0048	+.0183	.8795	+.0121	−.0266			
.6545		+.012	.8529	+.0134	−.0265			

(18)

The relation of Δq to q, based on these values, is shown as a broken line in figure 3. This curve differs only slightly from the solid line which shows the relation of Δq to q with the same average selective values of genotypes, but no sex difference. Thus the data are fitted substantially as well by both hypotheses.

It seemed next of interest to make the sex difference in selection value as great as possible in the homozygotes without ever exceeding the heterozygotes and subject to acceptance of the same average values, $W_{11}=.70$, $W_{12}=1.00$ and $W_{22}=.30$. This involves the assumption that CH/CH does not reproduce at all in one sex.

	Selection Value		
Genotype	W_f	W_m	W
ST/ST	1.00	.40	.70
ST/CH	1.00	1.00	1.00
CH/CH	.60	0	.30

(19)

In this case Δq approaches .25 as q approaches 0. The values of q and δq were calculated starting from q=0 and q=.99.

q	δq	Δq	q	δq	Δq
0	0	.25	.99	+.005	−.0070
.25	−.25	.1563	.9830	+.0070	−.0114
.4063	−.0938	.1070	.9715	+.0115	−.0177
.5133	−.0423	.0630	.9538	+ 0180	−.0256
.5763	−.0098	.0376	.9282	+.0263	−.0335
.6139	+.0070	.0228	.8947	+.0352	−.0391
.6367	+.0161	.0142	.8556	+.0426	−.0400
.6509	+.0213	.0089	.8157	+.0466	−.0363
.6598	+.0243	.0057	.7794	+.0468	−.0298
.6655	+.0262	.0037	.7496	+.0446	−.0227
.6692	+.0273	.0024	.7269	+.0413	−.0164
.6715	+.0280	.0015	.7106	+.0382	−.0114
.6731	+.0285	.0010	.6992	+.0356	−.008
.6741	+.0288	.0007			

(20)

A curve from these values is plotted in dot and dash in figure 3. It differs greatly from the solid line at both low and high values of q, but in the region represented by the observations (q=.292 to .663) it does not differ much.

There is therefore no reason from the observations at hand to rule out the possibility of very great selective differences between the sexes. It would require such experiments as the competitive mating of two types of males with each type of female and competitive mating of two types of females with each type of male to distinguish these hypotheses.

So far we have assumed that the relative selective values of the genotypes remain the same at all gene frequencies. This is not necessarily, or even probably, the case where selective mating is in question, and it becomes desirable to consider the consequences where the W's are functions of q. For simplicity we shall assume that sex differences are sufficiently small that they may be ignored. The general formula for Δq is as follows (WRIGHT 1942).

$$\Delta q = q(1-q)\sum W \frac{df}{dq} \Big/ 2\overline{W}, \qquad \overline{W} = \sum Wf. \tag{21}$$

If the W's, as reproductive rates, involve a function of q that is the same for all, this cancels in (21) leaving Δq the same as if the function were not involved. Thus populations with the same rates of change of gene frequencies may differ widely in the values of q at which the population reproduces most rapidly ($\overline{W}$ maximum), and these points are not in general at the point of equilibrium.

There is no such cancelling if the W's involve different functions of q. It must suffice here to illustrate by one hypothesis how widely the selective values may differ from those arrived at on the assumption that they are independent of q, and still fit the data.

Assume that the heterozygotes, instead of always being superior to both homozygotes, are always exactly intermediate in selective value, but also that the selective values of the homozygotes fall off linearly as the corresponding chromosome frequencies rise.

Genotype	*Frequency* (f)	*Selective Value* (W)	
ST/ST	q^2	$1 + a - bq$	
ST/CH	$2q(1-q)$	1	(22)
CH/CH	$(1-q)^2$	$1 - a + bq$	

$$\overline{W} = 1 - (a - bq)(1 - 2q) \tag{23}$$

$$\Delta q = q(1-q)(a - bq)/\overline{W} \tag{24}$$

$$\hat{q} = a/b \tag{25}$$

$$a - bq = \Delta q/[q(1 - q + (1 - 2q)\Delta q] \quad \text{from (23), (24)} \tag{26}$$

A rough determination of values of a and b to fit the observations under this hypothesis can be made by taking $\hat{q}$ as .70 (hence a=.70b from (25)) and substituting $\bar{q}$ and $\overline{\Delta q}$ in place of q and Δq in (26).

$$\begin{aligned} a &= .902 \\ b &= 1.288. \end{aligned} \tag{27}$$

Substitution of these values in (24) gives the dotted line in figure 3. This curve differs considerably from the solid line at low values of q, but differs

only slightly in the region covered by the observations and thus is a mathematically possible interpretation. How widely this hypothesis differs from that which fits the data on the hypothesis that selective values are independent of gene frequency may be seen from the following table.

Selective values (W)

	W's CONSTANT	W's VARY AS IN (22)						
		q=0	.10	.30	.50	.70	.90	1.00
ST/ST	.70	1.90	1.77	1.51	1.26	1.00	.74	.61
ST/CH	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
CH/CH	.30	.10	.23	.49	.74	1.00	1.26	1.39

An hypothesis of this nature is one that might well be approximated in nature in cases in which a species occupies a heterogenous environment. Each genotype may be favored by selection when rare and unable to occupy fully the ecological niches to which it is best adapted, but selected against when so abundant that it must in part occupy ecological niches to which it is less well adapted than other genotypes. This particular hypothesis is perhaps improbable under the conditions of the experiments, but the possibility that the W's depend on different functions of the gene frequencies is not improbable. This could be tested only by special experiments.

In the most extensive experiments, three types of chromosomes and consequently six genotypes were present. The analysis is much more complicated than where only two types of chromosome are present, and we shall restrict consideration to the hypothesis that selective values are independent of gene frequencies and that sex differences may be ignored.

As a first approach, the two chromosomes, Arrowhead and Chiricahua, which tended to decrease in frequency in the data, may be grouped together in opposition to Standard, which tended to increase in frequency. Let q refer to the frequency of Standard, s to its selective disadvantage when homozygous, and t to the selective disadvantage of Arrowhead and Chiricahua combined in comparison with the heterozygotes that involve Standard. Substitution in (8) yields

$$\begin{aligned} \overline{W} &= .7016 \text{ at } \bar{q} \\ s &= .555 \\ t &= .596 \\ \hat{q} &= .518. \end{aligned} \tag{29}$$

In this case, t is the weighted average of the selective disadvantages of AR/AR, AR/CH, and CH/CH relative to ST/AR and ST/CH combined. Using subscripts 1, 2, and 3 for Standard, Arrowhead, and Chiricahua, respectively, and indicating selective disadvantages of genotypes by double symbols, we may write

$$t = .596 = (s_{22}q_2^2 + 2s_{23}q_2q_3 + s_{33}q_3^2)/(1 - q_1)^2. \tag{30}$$

If now it be assumed that the heterozygote AR/CH is at no selective disadvantage relative to the other heterozygotes (that is, $s_{23}=0$), we can estimate roughly the average selective disadvantages of the AR/AR and CH/CH by putting these the same in the above equation and substituting $\bar{q}_1=.4201$, $\bar{q}_2=.3182$ and $\bar{q}_3=.2617$ (from table 10) for q_1, q_2, and q_3, respectively. But this yields the impossible value, $s_{22}=s_{33}=1.17$ which implies that AR/AR and CH/CH suffer fates 17 percent worse than death on the average.

This lumping together of different genotypes however, is not a very satisfactory procedure, especially since it is obvious from the data that Arrowhead and Chiricahua do not react alike. Consider next the hypothesis that the three heterozygotes are equivalent in selective value ($W_{12}=W_{13}=W_{23}=1$) but that the homozygotes suffer diverse selective handicaps (s_1, s_2 and s_3).

Genotype	*Frequency*	*Selective Value*	
ST/ST	q_1^2	$1-s_1$	
ST/AR	$2q_1q_2$	1	
ST/CH	$2q_1q_3$	1	(31)
AR/AR	q_2^2	$1-s_2$	
AR/CH	$2q_2q_3$	1	
CH/CH	q_3^2	$1-s_3$	

$$\overline{W} = 1 - s_1q_1^2 - s_2q_2^2 - s_3q_3^2 \tag{32}$$

$$\Delta q_1 = q_1\left[\frac{1-s_1q_1}{\overline{W}} - 1\right] \tag{33}$$

$$\Delta q_2 = q_2\left[\frac{1-s_2q_2}{\overline{W}} - 1\right]$$

$$\Delta q_3 = q_3\left[\frac{1-s_3q_3}{\overline{W}} - 1\right]$$

$$\frac{\partial \overline{W}}{\partial q_1} = -2\left[s_1q_1 + s_2q_2\frac{\partial q_2}{\partial q_1} + s_3q_3\frac{\partial q_3}{\partial q_1}\right]. \tag{34}$$

$$\frac{\partial \Delta q_1}{\partial q_1} = \frac{\Delta q_1}{q_1} - q_1\left\{\overline{W}s_1 + (1-s_1q_1)\frac{\partial \overline{W}}{\partial q_1}\right\}\Big/\overline{W}^2. \tag{35}$$

After some reduction and noting that since $q_1+q_2+q_3=1$, $1+\frac{\partial q_2}{\partial q_1}+\frac{\partial q_3}{\partial q_1}=0$

$$\frac{\partial \Delta q_1}{\partial q_1} = 1 - \frac{1}{\overline{W}} + 2\frac{\Delta q_1}{q_1} - 2q_1\left(1+\frac{\Delta q_1}{q_1}\right)\left(\frac{\Delta q_1}{q_1}+\frac{\Delta q_2}{q_2}\frac{\partial q_2}{\partial q_1}+\frac{\Delta q_3}{q_3}\frac{\partial q_3}{\partial q_1}\right). \tag{36}$$

Assuming that all observed values of q are in the region in which relations are approximately linear, an approximate value of $\overline{W}$ may be obtained by replacing the q's and Δq's by their mean values and replacing $\frac{\partial q_2}{\partial q_1}$ by

the regression coefficient $b_{q_2q_1}(=-.2657)$, $\frac{\partial q_3}{\partial q_1}$ by $b_{q_3q_1}(=-.7343)$ and $\frac{\partial \Delta q_1}{\partial q_1}$ by $b_{\Delta q_1 q_1}(=-.3865)$. With these assumptions, $\overline{W}$ comes out .7091 for the set of mean gene frequencies. The values of s_1, s_2, and s_3 can now be estimated form (33).

$$\begin{aligned} s_1 &= .536 & W_{11} &= .464 \\ s_2 &= 1.000 & W_{22} &= .000 \\ s_3 &= 1.389 & W_{33} &= -.389. \end{aligned} \tag{37}$$

The estimated value of s_1 (.536) does not differ much from that obtained by lumping Arrowhead and Chiricahua (.518). The selection against AR/AR is complete and that against CH/CH is impossible, 38.9 percent greater than complete elimination. The weighted average selective disdvantage of AR/AR, AR/CH and CH/CH comes out .584, in approximate agreement with .596 where these were lumped. It appears to be impossible to account for the results on the hypothesis that the heterozygotes are all equivalent. The selective values must be allowed more latitude.

Genotypes	*Frequencies*	*Selective Values*
ST/ST	q_1^2	W_{11}
ST/AR	$2q_1q_2$	W_{12}
ST/CH	$2q_1q_3$	W_{13}
AR/AR	q_2^2	W_{22}
AR/CH	$2q_2q_3$	W_{23}
CH/CH	q_3^2	W_{33}

(38)

$$\overline{W} = W_{11}q_1^2 + 2W_{12}q_1q_2 + 2W_{13}q_1q_3 + W_{22}q_2^2 + 2W_{23}q_2q_3 + W_{33}q_3^2 \tag{39}$$

$$\begin{aligned} \Delta q_1 &= q_1\left[\frac{W_{11}q_1 + W_{12}q_2 + W_{13}q_3}{\overline{W}} - 1\right] \\ \Delta q_2 &= q_2\left[\frac{W_{12}q_1 + W_{22}q_2 + W_{23}q_3}{\overline{W}} - 1\right] \\ \Delta q_3 &= q_3\left[\frac{W_{13}q_1 + W_{23}q_2 + W_{33}q_3}{\overline{W}} - 1\right]. \end{aligned} \tag{40}$$

At equilibrium $\Delta q_1 = \Delta q_2 = \Delta q_3 = 0$

$$\begin{aligned} W_{11}\hat{q}_1 + W_{12}\hat{q}_2 + W_{13}\hat{q}_3 - \overline{W} &= 0 \\ W_{12}\hat{q}_1 + W_{22}\hat{q}_2 + W_{23}\hat{q}_3 - \overline{W} &= 0 \\ W_{13}\hat{q}_1 + W_{23}\hat{q}_2 + W_{33}\hat{q}_3 - \overline{W} &= 0. \end{aligned}$$

$\overline{W}$ with its quadratic terms can be eliminated, leaving a series of linear equations to solve for two variables $\hat{q}_1$ and $\hat{q}_2$ (noting that $\hat{q}_3 = 1 - \hat{q}_1 - \hat{q}_2$). An analogous system of $(n-1)$ simultaneous linear equations would appear with n instead of three alternatives. In the present case let

$$K_1 = W_{22}W_{33} - W_{13}W_{22} - W_{12}W_{33} + W_{12}W_{23} + W_{13}W_{23} - W_{23}^2$$
$$K_2 = W_{33}W_{11} - W_{12}W_{33} - W_{13}W_{11} + W_{23}W_{13} + W_{12}W_{13} - W_{13}^2 \quad (42)$$
$$K_3 = W_{11}W_{22} - W_{23}W_{11} - W_{13}W_{22} + W_{13}W_{12} + W_{23}W_{12} - W_{12}^2.$$

Then

$$\hat{q}_1 = K_1/(K_1 + K_2 + K_3)$$
$$\hat{q}_2 = K_2/(K_1 + K_2 + K_3) \quad (43)$$
$$\hat{q}_3 = K_3/(K_1 + K_2 + K_3).$$

It may be noted that in the special case considered earlier in which the selective values of all heterozygotes are 1, the condition that $\Delta q_1 = \Delta q_2 = \Delta q_3 = 0$ gives at once $s_1q_1 = s_2q_2 = s_3q_3 = 1 - \overline{W}$ which gives

$$\hat{q}_1 = \frac{1}{s_1} \Big/ \sum \frac{1}{s} \quad (44)$$

with analogous formulae for $\hat{q}_2$, $\hat{q}_3$ (and for other q's if there are more than three alternatives).

Returning to the case in which no restriction (other than constancy) are placed on the W's, the observation equations are as follows (from 40)

$$q_1 + \Delta q_1 = (q_1^2W_{11} + q_1q_2W_{12} + q_1q_3W_{13})/\overline{W}$$
$$q_2 + \Delta q_2 = (q_1q_2W_{12} + q_2^2W_{22} + q_2q_3W_{23})/\overline{W} \quad (45)$$
$$q_3 + \Delta q_3 = (q_1q_3W_{13} + q_2q_3W_{23} + q_3^2W_{33})/\overline{W}.$$

It is convenient to let $y_1 = q_1 + \Delta q_1$, $y_2 = q_2 + \Delta q_2$, $y_3 = q_3 + \Delta q_3$, $x_{11} = q_1^2/\overline{W}$, $x_{12} = q_1q_2/\overline{W}$ etc. The quantity to be minimized is then

$$\sum \delta^2 = \sum [(y_1 - x_{11}W_{11} - x_{12}W_{12} - x_{13}W_{13})^2]$$
$$+ \sum [(y_2 - x_{12}W_{12} - x_{22}W_{22} - x_{23}W_{23})^2] \quad (46)$$
$$+ \sum [(y_3 - x_{13}W_{13} - x_{23}W_{23} - x_{33}W_{33})^2].$$

The six normal equations with the unknown selective values written for convenience above are as follows:

W_{11}		W_{12}		W_{13}		W_{22}		W_{23}		W_{33}		
$\sum x_{11}^2$	+	$\sum x_{11}x_{12}$	+	$\sum x_{11}x_{13}$	+	0	+	0	+	0	=	$\sum x_{11}y_1$
$\sum x_{11}x_{12}$	+	$2\sum w_{12}^2$	+	$\sum x_{12}x_{13}$	+	$\sum x_{12}x_{22}$	+	$\sum x_{12}x_{23}$		0	=	$\sum x_{12}y_1 + \sum x_{12}y_2$
$\sum x_{11}x_{13}$	+	$\sum x_{12}x_{13}$	+	$2\sum x_{13}^2$	+	0		$\sum x_{13}x_{23}$	+	$\sum x_{13}x_{33}$	=	$\sum x_{13}y_1 + \sum x_{13}y_3$
0	+	$\sum x_{12}x_{22}$	+	0	+	$\sum x_{22}^2$	+	$\sum x_{22}x_{23}$	+	0	=	$\sum x_{22}y_2$
0	+	$\sum x_{12}x_{23}$	+	$\sum x_{12}x_{23}$	+	$\sum x_{22}x_{23}$	+	$2\sum x_{23}^2$	+	$\sum x_{23}x_{33}$	=	$\sum x_{23}y_2 + \sum x_{23}y_3$
0	+	0	+	$\sum x_{13}x_{33}$	+	0	+	$\sum x_{23}x_{33}$	+	$\sum x_{33}^2$	=	$\sum x_{33}y_3$.

These could be reduced to five, taking one of the W's as standard, since only the relative values are in question. The iteration process necessitated by the use of trial values of the W's in estimating $\overline{W}$ for each observation however, is facilitated by allowing all of the W's to vary. As before, no attempt was made at differential weighting of the observations.

The initial trials were based on the estimates of $W_{11}=(.696)$, $W_{33}(=.305)$, with $W_{13}(=1.000$ as standard), deduced from the experiments with Standard and Chiricahua only, and on preliminary deductions for W_{12}, W_{23} and W_{22}. These trial values turned out to be unsatisfactory, but approximate stability was reached at the fourth iteration. The trial values (used in $\overline{W}$) and the results from solution of the normal equations were as follows:

	1ST TRIAL		2ND TRIAL		3RD TRIAL		4TH TRIAL		FINAL ESTIMATE
	TRIED	CALC.	TRIED	CALC.	TRIED	CALC.	TRIED	CALC.	$W_{13}=1$
W_{11}	.696	.636	.656	.622	.60	.594	.59	.597	.43
W_{12}	2.027	1.911	1.950	1.868	1.76	1.785	1.79	1.797	1.30
W_{13}	1.000	1.281	1.189	1.347	1.44	1.411	1.41	1.394	1.00
W_{22}	.305	.034	.125	.053	.06	.081	.08	.073	.05
W_{23}	.965	.969	.968	.980	.96	.984	.98	.982	.71
W_{33}	.305	.388	.361	.340	.35	.275	.28	.284	.21

While complete stability is not reached, the values given in the last column (reduced to a scale on which $W_{13}=1.00$) should be sufficiently accurate for the hypothesis under consideration. The reduction in the selective value of ST/ST from .696 to .43 and for CH/CH from .305 to .21 suggests increased selection against the homozygotes in the presence of three chromosomes instead of two, or, in other words, that the selective values are to some extent at least functions of the chromosome frequencies instead of constants as here assumed. The method, however, does not yield any impossible estimates of W, although the selection against homozygous Arrowhead is indicated to be almost complete.

The frequencies of the three chromosomes at equilibrium can be calculated from the above estimates of the W's by substitutions in (42) and (43).

Standard	$\hat{q}_1=.531$
Arrowhead	$\hat{q}_2=.339$
Chiricahua	$\hat{q}_3=.130$

DISCUSSION

There can be no doubt from these experiments that the three chromosome types found in the population of Piñon Flats, Mount San Jacinto, are not alike in selective value, even though the data do not yield a unique interpretation of the selective processes that take place. Under the conditions obtaining in the experimental populations, the selective values are such that a certain set of frequencies of chromosome types is in equilibrium. Furthermore, the selective values are quite different under different conditions, temperature being at least one of the modifying agents. In a general way, this makes the observation that there are marked seasonal changes in the frequencies of the chromosome types in natural populations understandable. But a detailed application of the experimental results to the interpretation of the changes observed in nature encounters difficulties.

Parallel cyclic changes occur in the populations of Piñon Flats (4000 feet above sea level) and Andreas Canyon (800 feet). In both localities the

frequencies of Standard are lowest and of Chiricahua highest in June. The waning July population at Piñon shows some increase of Standard and a drop in Chiricahua; this process continues till September when Standard about reaches its annual maximum frequency and Chiricahua its minimum. Flies are rare in July and August at Andreas, but in September the population shows a materially increased frequency of Standard and a decreased one of Chiricahua. By analogy with the populations of experimental cages kept at 25°C, it is tempting to ascribe the waxing of Standard and the waning of Chricahua at Piñon and Andreas to the high temperatures prevailing during the summer months in these localities. The same analogy seems to account for the fact that Standard chromosomes are less frequent and Chiricahua more frequent at Piñon than in the warmest locality, Andreas. Furthermore, at Keen Camp (about 4500 feet elevation, ponderosa pine zone) Standard is still less and Chiricahua still more frequent than at Piñon (DOBZHANSKY 1943).

Again, no appreciable changes in the frequencies of the chromosomal types take place in any locality during the cool season, from September to March or April. This agrees with the finding that chromosome frequencies change little if at all in experimental cages kept at 16½°C. Although Keen is the coolest of the three localities, a similar explanation is hardly satisfactory to account for the absence at Keen of the cyclic changes of the type observed at Piñon and Andreas. The selective values of the chromosomes at Keen (and Andreas) need not be the same as those at Piñon.

The frequencies of Standard drop and of Chiricahua increase from April to June at Piñon and at Andreas. Since the temperatures are increasing during spring, the observed changes in the chromosome frequencies go in the direction opposite to that found at high temperatures in the experimental populations. The causation of these changes is at present a matter of speculation.

The sensitivity of the selective differentials to environmental changes is probably the most interesting phenomenon observed in our experiments, apart from the establishment of the fact that chromosome types found in natural populations may differ in adaptive value. As shown above, the chromosome types are equivalent or nearly so at 16½°C, but strikingly unlike at higher temperatures. An analogous extreme sensitivity to temperature has been found also in homozygotes for some individual chromosomes isolated from natural populations (DOBZHANSKY and SPASSKY 1944). It must be kept in mind that, among the mass of possible environmental variables, only temperature and the amount of food have so far been studied in population cages. It seems very improbable that the relative adaptive values of the chromosome types so greatly modified by temperature will prove to be insensitive to anything else. The changes observed at Piñon and Andreas during spring may, then, represent a selective response to factors other than temperature (for example, qualitative changes in the diet). By the same token, the causative factor of the changes taking place in the same populations in summer need not necessarily be temperature, even though temperature does induce analogous changes in population cages.

An alternative explanation has been suggested by HOVANITZ (1944)—namely, that the populations of Piñon and Andreas are swamped in summer by migration from the flourishing populations higher on the mountain, which resemble Keen in chromosome frequencies. The return of Piñon and Andreas in late summer and in fall to the frequencies of the chromosome types characteristic for the winter populations of these localities obviously cannot be accounted for by migration. It must be due to selective pressures of the sort indicated by the experiments with population cages, or even to complete extinction of the migrant population which must somehow escape crossing with the natives. Furthermore, the amount of dispersion as determined in *Drosophila pseudoobscura* by experiments in the field is not adequate (DOBZHANSKY and WRIGHT 1943). The mean square radial distance in one day from the site of release of dense populations of marked flies was 17,600m² with variance of distances increasing on subsequent days by about 8000m² per day at 72°F. The dispersion variance (close to zero below 60°F) increased by about 760m² for each increment of one degree Fahrenheit. These figures must be halved to give the variance of spread in one direction. Thus even assuming a temperature of 93°F, the increment of variance in a given direction of flies that emerge at one place on a certain day and spread by random flights is only about 12,000m² per day. In a month, the standard deviation of the group about its point of origin would be only about 600 meters. In the experiments moreover, there was evidence that about half of the flies released died in a week. Since Andreas is about 16 kilometers from Keen down the mountain, it is obvious that random dispersal from Keen at the observed rate is wholly inadequate to account for the rapid shift at Andreas to a Keen-like composition of the population. The only possibility of accounting for the May–June shift at Piñon and Andreas by migration from higher on the mountain seems to be by a directed mass movement at a rate of at least half a kilometer per day.

Cyclic changes in the composition of populations have been discovered by TIMOFEEFF-RESSOVSKY (1940) in the beetle *Adalia bipunctata*, by GERSHENSON (1945) in the hamster *Cricetus cricetus*, and by DUBININ and TINIAKOV (1945) in *Drosophila funebris*. The changes appear to be caused in all cases by natural selection in response to seasonal alterations in the environments. The situation found in *Drosophila pseudoobscura* is, thus, not unique. It furnishes, however, the only instance in which changes analogous to those taking place in natural populations can be in part reproduced in laboratory experiments.

SUMMARY

Artificial populations of *Drosophila pseudoobscura* were kept in cages constructed as a modification of the model devised by L'HÉRITIER and TEISSIER.

All the flies used in the experiments reported in this paper are descendants of parents collected at Piñon Flats, Mount San Jacinto, California. Three types of third chromosomes, Standard, Chiricahua, and Arrowhead, are common in the population of that locality. Their relative frequencies undergo

cyclic seasonal changes—namely, Standard increases and Chiricahua decreases in frequency during the summer; the opposite change takes place during spring; the relative frequencies remain constant during fall and winter.

Most of the experiments began with approximately equal numbers either of two or of three chromosome types. At 25°, with initial frequencies equal or with Chiricahua more frequent than Standard, there was a highly significant increase in frequency of Standard (where present) at the expense of either of the other types but especially of Chiricahua. On the other hand, there were no significant changes in mean frequency at 16.5°C. The rate of change at 25° showed a strong negative regression on frequency in all cases, and a probably significant regression of this sort was observed in one case (Arrowhead) at 16.5°. The data further indicate that all chromosome types are favored when sufficiently rare and opposed when sufficiently common.

The simplest hypothesis is that the heterozygotes are favored over both corresponding homozygotes and that there are no sex differences. The results in the experiments at 25° in which only Standard and Chiricahua were present can be adequately explained by postulating the relative selective values .70:1.00:.30 for ST/ST, ST/CH and CH/CH, respectively. Equilibrium is indicated at 70 percent Standard, 30 percent Chiricahua.

However, there is no assurance that selection acts alike on males and females. The mathematical theory for unequal selective values in the sexes is presented. It is found that the results differ little except in extreme cases from those found with sex equality in selection. Thus the data can be fitted substantially as well by assuming selective values 1.00:1.00:.60 for females, .40:1.00:0 for males (for ST/ST, ST/CH, and CH/CH, respectively) as under the previous hypothesis in which both sexes are selected according to the averages of those figures.

It is possible that the selective values may vary with the changes in composition of the population. The mathematical theory of variable selection coefficients is discussed briefly. An extreme hypothesis of this sort (heterozygotes always intermediate while each homozygote decreases in selective value with increase in frequency) was found to fit the data reasonably well. The selective values arrived at were $(1.90-1.29q)$ for ST/ST, 1.00 for ST/CH and $(.10+1.29q)$ for CH/CH, where q is the frequency of Standard (.70 at equilibrium as before).

The mathematical theory of selection among multiple alleles is considered in connection with the cases in which three chromosome types were present. The selective values at 25°, indicated by the method of least squares, are .43 for ST/ST, .05 for AR/AR, .21 for CH/CH, 1.30 for ST/AR, 1.00 for ST/CH, and .71 for AR/CH. Equilibrium is indicated at 53 percent Standard, 34 percent Arrowhead and 13 percent Chiricahua. The hypothesis that all three heterozygotes are equal in selective value gives impossible results.

The experimental results demonstrate clearly that there may be selective differences between chromosome types derived from the same locality and that

these may be of such a nature as to result in the indefinite persistence of several types in such a locality. The marked rise in frequency of Standard and decrease of Chiricahua in summer, observed to occur in nature in the locality from which the flies were collected, is analogous to the experimental reaction to high temperature, and it is tempting to compare the lack of change in artificial population at 16.5° with the constancy observed in the natural ones during fall and winter. The changes which occur in nature during spring (the reverse of those in summer) however, have not been reproduced in the population cages. Mass migration at this time ffom the population high in the mountains would explain the results qualitatively, but it would be difficult to reconcile this with the results of earlier experiments on the rate of dispersion of the flies in nature. It appears that there are important factors yet to be discovered.

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AN ANALYSIS OF TRANSLOCATIONS IN THE MOUSE

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INTRODUCTION

RADIATION-INDUCED translocations in the mouse have been studied by SNELL, BODEMANN, and HOLLANDER (1934), SNELL (1935, 1939, 1941a, 1941b), SNELL and PICKEN (1935), SNELL and AMES (1939), HERTWIG (1938, 1940), KOLLER and AUERBACH (1941), and KOLLER (1944). The results of SNELL and co-workers have been summarized elsewhere (1941a).

HERTWIG (1940) has made a very thorough and detailed investigation of prenatal mortality, litter size, and inheritance of semi-sterility in 11 out of a total of 60 semi-sterile lines derived from irradiated males. The prenatal mortality was determined from sections of the uteri and ovaries of 300 normal females mated to semi-sterile males and killed ten days or less post-coitus. The sectioned uteri and ovaries of 211 normal females×normal males served as controls. Embryos were classified as normal, dead embryos, large implantations (that is, large uterine swellings containing only the remains of an embryo), and small implantations. A corpus luteum count indicated the number of ova fertilized, or at least available for fertilization. In the semi-sterile group, the embryos showed 36.10±0.91 percent survival (number of normal embryos×100/number of corpora lutea); in the control group the figure was 75.37±0.99 percent. Hence the fertility of the test animals was slightly less than 50 percent of the fertility of the controls.

The fertility of the test animals may be calculated also from the size of litters carried to term. The mating of semi-steriles, both males and females, from 11 translocation stocks,×normals gave 1462 litters with a mean litter size of 3.12±0.002. Comparable normal matings gave 790 litters with a mean litter size of 6.86±0.064. The fertility of the translocation heterozygotes is, therefore, 45.48 percent that of normal mice. Again the fertility is reduced by slightly more than one-half. This is the average figure for the 11 different stocks. Taking the stocks separately, the fertilities for the different translocations each in terms of its control are: 41.8 percent, 42.9 percent, 44.2 percent, 45.2 percent, 45.7 percent, 46.3 percent, 48.1 percent, 49.1 percent, 50.6 percent, 52.2 percent, 58.7 percent. If these figures are broken down still further according to which parent is the heterozygote and to whether, in the case of male heterozygotes, the female is a sister or unrelated, there is seen to be a considerable lack of consistency in the data. Hence the significance of the individual percentages is somewhat open to question. Nevertheless, a tendency for at least some translocations in the mouse to cause a reduction in fertility to something less than 50 percent of normal is very strongly indicated.

The litter size for the mating translocation heterozygote×translocation heterozygote was also investigated and found to be 37.17 percent of normal (average figure for 11 stocks).

From the mating heterozygote×normal, 1031 young were raised and tested for semi-sterility. The expected proportion of semi-sterile offspring is one-half. The observed figures were considerably less than this—namely, 31.6 percent to 50.6 percent for the different stocks, with a mean for all stocks of 43.9 percent. The number of litters raised in testing individual mice for semi-sterility or normal fertility is not indicated, nor is the mean litter size taken as the dividing line between the two groups. As an explanation for this deficiency of translocation heterozygotes, HERTWIG suggests that they are subject to a greater prenatal mortality than are homozygous normals. The mortality of this class of zygotes is in turn assumed to explain the reduction of litter size, where one parent is a translocation heterozygote, to less than one-half normal.

An attempt to obtain translocation homozygotes from six of the semi-sterile stocks was successful in two cases and probably successful in a third. Since the number of animals tested in each case was quite limited, it cannot be concluded that the other three stocks were incapable of yielding homozygotes.

The embryological material presented by HERTWIG will be summarized in a later paper.

KOLLER (1944) made a cytological and genetical analysis of three radiation induced semi-sterile lines in mice. On the basis of rather limited data, the fertilities of the three lines are found to be: line A, 29.9–39 percent of normal; line B, 45.7 percent; line T, 43.6–46.5 percent. An interesting correlation is found between the fertility percentage and the types of chromosome configurations observable at first meiotic metaphase. These configurations are classified as: disjunctional ring-of-four, non-disjunctional ring-of-four, disjunctional chain-of-four, non-disjunctional chain-of-four, normal figure-of-eight ("the result of five crossovers, one of which has taken place in an interstitial segment—that is, in the chromosome region between a centromere and the point of exchange,—while the four others are formed in the interchange segments and in the short arms"), abnormal figure-of-eight (similar to a normal figure-of-eight but showing non-disjunction of the centromeres), association-of-three, unequal bivalent, univalent, non-analyzed. Line A shows a particularly high proportion of non-disjunctional rings- and chains-of-four, of abnormal figures-of-eight, and of univalents, all of which produce all or predominantly unbalanced gametes. KOLLER concludes: "Chromosomes of the mouse are too small and numerous to enable very detailed and accurate observations to be made. The data which have been obtained are sufficient, however, to show that the differences in fertility between the lines are correlated with the frequency of disjunctional co-orientation of chromosomes within the translocation ring and may also be influenced by differences in the frequency of associations-of-three and of univalents."

WALETZKY and OWEN (1942) have described a partially sterile condition which appeared spontaneously in a single male rat and was transmitted to half his offspring. Normal females mated to partially sterile males and killed before term showed the same types of dead and abnormal embryos that are found in translocation stocks in mice.

STOCKS AND NOMENCLATURE

Of a number of translocations induced by the X-irradiation of males, the X-irradiation of females, and the neutron-irradiation of males, six were selected for detailed study. These are: *T(5;8)a* (previously called *T-F1146*) from an X-irradiated male, *T(1;?)c* (previously called *T-R112*) from a neutron irradiated male, *T(2;?)d* (previously called *T-R158*) from a neutron-irradiated male, *T-F1111* and *T-F1194* from X-irradiated females, and *T-R18* from a neutron-irradiated male. These translocations originally were designated by *T-* followed by the number of the mouse in which they appeared. More recently, where one or both of the chromosomes involved in a translocation has been identified, the original symbol has been replaced by the type of designation used in Drosophila (BRIDGES and BREHME 1944). The numbers in parenthesis are the numbers of the chromosomes (as assigned by the Committee on Mouse Genetics Nomenclature, DUNN, GRUNEBERG, and SNELL 1940) involved in the translocation. A question mark in place of one of the numbers indicates that one of the chromosomes is unknown. The final letter is the known gene closest to the break.

A word is in order in regard to the chromosomes designated 8 and 9. The highest number assigned by the Committee on Mouse Genetics Nomenclature was 7. DUNN and CASPARI (1942) described a new linkage group comprising the genes *T*, *Ki*, and *Fu*. HERTWIG (1942) described a new linkage group comprising the genes *an* (Anamie) and *b* (brown) and assigned it the number 8. WOOLLEY (1943) reported a linkage between *m* (misty) and *b*, and, because HERTWIG's paper had not reached this country, assigned it, at the writer's suggestion, the number 9, treating the *T*, *Ki*, *Fu* group as 8. It would now appear that the best usage was to call the *an*, *b*, *m* group, chromosome 8, and this usage will be followed here.

There is also confusion in regard to the symbol *m*. WOOLLEY (1941) used this symbol for the new mutant "misty"; in 1942 HERTWIG assigned it to the mutation "mikrophthalmus." Its usage to denote misty has priority, and we shall employ it for this gene.

The following multiple factor stocks were used in linkage tests: CA stock: *CaCa Fufu Ww;* P stock: *aa bb dse/dse pp;* V stock: *aa lnln ss wa-1wa-1 vv;* W stock: *ff sh-2 wa-2/sh-2 wa-2*.

The P stock is also *rr*, but this locus was not used in the tests.

To test males for semi-sterility, matings were usually made to females of the B alb C stock. This stock, originally developed by MACDOWELL, is particularly suited to this purpose because of the consistent and uniform fertility of the females.

METHODS

Pregnant females were isolated in separate pens and examined, with occasional exceptions, daily. Litters were thus usually recorded within 24 hours of birth.

The classification of mice as semi-sterile or normal on the basis of the mean size of litters produced is usually quite clear but occasionally involves a con-

TABLE 1

Linkage tests with three translocations; backcross mice tested for semi-sterility.

TRANSLOCATION	MUTATION		++	x+	+T	xT	TOTAL	CROSSOVER PERCENTAGE
T(5;8)a	*a*	repulsion	8	0	0	20	28	0.0
	b	coupling	6	16	20	6	48	25.0
	Ca	repulsion	4	5	8	4	21	38.1
	Ca	coupling	2	9	10	11	32	59.4
	f	coupling	6	6	6	2	20	40.0
	Fu	repulsion	2	7	7	5	21	33.3
	In	coupling	3	4	6	8	21	52.4
	p	repulsion	5	3	10	10	28	46.4
	s	coupling	3	4	6	8	21	52.4
	sh-2	coupling	6	6	6	2	20	40.0
	v	coupling	5	2	10	4	21	42.8
	W	repulsion	6	3	7	5	21	52.4
	wa-1	coupling	5	1	7	8	21	61.9
	wa-2	coupling	6	6	5	3	20	45.0
T(1;?)c	*a*	coupling	7	9	7	8	31	48.4
	b	repulsion	5	5	3	2	15	53.3
	Ca	repulsion	8	7	1	5	21	61.9
	d	coupling	3	3	2	5	13	61.5
	f	coupling	6	2	7	4	19	52.6
	Fu	repulsion	8	7	0	5	20	65.0
	*je**	coupling	4	2	1	2	9	66.7
	*je**	F_2 coupling	51	21	19	4	95	41.3
	In	coupling	4	0	5	3	12	61.7
	p	coupling	2	4	6	1	13	23.1
	*Re**	coupling	24	15	25	25	89	44.9
	*Re**	repulsion	16	12	24	10	62	41.9
	s	coupling	3	1	6	2	12	41.7
	sh-2	coupling	5	3	5	6	19	57.9
	*Sp**	repulsion	18	10	25	9	62	43.6
	v	coupling	4	0	6	2	12	50.0
	W	repulsion	6	9	3	2	20	40.0
	wa-1	coupling	2	2	4	4	12	50.0
	wa-2	coupling	2	6	5	6	19	42.1
T(2;?)d	*a*	coupling	3	3	0	5	11	72.7
	*b***	repulsion	6	6	2	2	16	50.0
	Ca	repulsion	2	3	3	1	9	33.3
	*Ca***	repulsion	11	9	7	7	34	47.0
	d	coupling	0	12	9	2	23	8.7
	d†	coupling	3	4	22	5	34	23.5
	f	coupling	6	6	6	3	21	42.8
	Fu	repulsion	3	2	2	2	9	55.6
	In	coupling	3	8	4	6	21	42.8
	p	coupling	3	9	5	6	23	39.1
	s	coupling	5	6	4	6	21	52.4
	sh-2	coupling	6	6	7	2	21	38.1

TABLE 1—*Continued*

TRANSLOCATION	MUTATION		++	x+	+T	xT	TOTAL	CROSSOVER PERCENTAGE
	v	coupling	8	3	7	3	21	52.4
	W	repulsion	2	2	2	1	7	42.8
	wa-1	coupling	5	6	5	5	21	47.6
	wa-2	coupling	3	9	6	3	21	28.6
	*wa-2***	coupling	14	12	10	13	49	44.9

* Presence or absence of $T(1;?)c$ in backcross (or F_2) animals was determined by presence or absence of *c*, which was segregating in this cross and with which $T(1;?)c$ is very closely linked, and not by tests for semi-sterility. Coupling is used to refer to the relationship in which $(T1;?)c$ and the gene under test entered the cross. When this gene was recessive (that is, *je*), the relationship with *c* was actually one of repulsion.

** Presence or absence of $T(2;?)d$ in backcross animals was determined (with the probability of some error) by the presence or absence of *d*, which was segregating in this cross and with which $T(2;?)d$ is linked, and not by tests for semi-sterility.

† These animals are from litters from which most dilute mice were discarded without testing for semi-sterility. The animals were less thoroughly tested than in the cross listed above so that there is greater chance of error in the classification.

siderable element of judgment. Males are more easily classified than females; the latter, even when genetically normal, occasionally throw a succession of small litters due to unknown causes. Four or more litters were usually raised from each mouse. In the case of important and doubtful males, 12 or more litters were sometimes raised. In uncertain cases, the age, general condition, presence of detrimental recessives, and probable hybrid vigor of females were taken into consideration as well as the mean litter size. Mice throwing litters averaging less than 4.6 were always classed as semi-sterile; mice throwing litters of more than 6, as normal; within the range 4.6 to 6 the element of judgment entered (table 9).

LINKAGES

For initial linkage tests, semi-sterile mice of the six translocation stocks were crossed to mice of the CA, P, V and WA stocks, and a backcross raised and tested for semi-sterility. About 20 backcross animals were usually tested. This gave tests for a large number of combinations (six translocations tested against 15 genes on 12–13 chromosomes) but yielded insufficient data for the detection of anything except close linkages. This method was selected because loose linkages would not lend themselves to further tests, whereas close linkages would do so. In the case of $T(1;?)c$, tests were also made with *je*, *Re* and *Sp* (*Sp*=splotch, a new dominant spotting mutation, W. L. Russell, unpublished).

In the case of the translocations $T\text{-}F_1111$, $T\text{-}F_1194$, and $T\text{-}R_18$ no clear linkages were found. $T\text{-}R_18$ showed only 25 percent crossing over (24 animals) with *W*, suggesting a loose linkage, but because $T\text{-}R_18$ showed a higher mean litter size than any of the other translocations, making it difficult to work with, this cross was not pursued further. Other crossover percentages ranged

from 42.1 to 70.0 in the case of *T-F*$_1$*111*, from 38.9 to 61.2 in the case of *T-F*$_1$*194*, and from 27.8 to 68.4 in the case of *T-R*$_1$*8*. Since clear linkages were not found, these translocation stocks were discarded. (A linkage between *T-F*$_1$*111* and *wa-2* reported in a preliminary communication (SNELL 1941b) as probable but requiring more data was not substantiated by further tests.)

The other three translocations all proved to involve tagged chromosomes (table 1). *T(5;8)a* gave no crossovers with *a*, 25 percent crossovers with *b*. *T(1;?)c* gave 23.1 percent crossovers with *p*. *T(2;?)d* gave 8.7 percent crossing over with *d* in one test, 23.5 percent in a second test.

Tests were next set up with translocations *T(5;8)a* and *T(1;?)c* involving the other known genes on chromosomes 5, 8, and 1.

The mating *Pa a T(5;8)a B/pa A+b*×*pa a+b/pa a+b* gave the results shown in table 2. The crossover value between *pa* and *a* was 19.8±3.8 percent for the female and 15.8±3.3 percent for the male (table 5). This compares with normal values of 21.2±2.7 percent and 19.6±2.6 percent, respectively (ROBERTS and QUISENBERRY 1935). The crossover value between *a* and *b* was 31.0±4.3 percent in the female, 25.3±3.6 percent in the male. These genes show no linkage in the normal mouse.

TABLE 2

Segregants from the mating +a T(5;8)a+/pa++b×*pa a b/pa a b.*

HETEROZYGOUS SEX	*+a+*	*pa+b*	*pa a+*	*++b*	*+a b*	*pa++*	*pa a b*	*+++*	TOTAL
Female	39	27	3	11	8	19	4	5	116
Male	48	42	8	11	16	17	2	2	146

The crosses listed in table 1 showed zero crossovers between *a* and semi-sterility in a total of 28 mice, establishing *a* as close to the break in chromosome 5. The female was the heterozygous parent in this case. Further data were obtained by testing for semi-sterility mice of the phenotypes *+a b*, *pa ++*, *pa a b*, and *+++* from the cross discussed in the previous paragraph. Table 2 shows 73 mice in these four classes. Tests were completed on 53 of these, showing one male, No. To1255, to be a crossover of the genotype *pa A T(5;8)a B/pa a+b*. This male was from a mating in which the male was the heterozygous parent. He gave 12 litters averaging 4.3 young and transmitted semi-sterility to his descendants, the semi-sterility remaining associated with *A* except in the case of one daughter which was apparently a second crossover (one out of 11 possible cases). Allowing for the untested mice of the four classes named above, we may estimate a frequency of zero crossovers in 77 mice where the female was the heterozygous sex, one crossover in 114 mice where the male was the heterozygous sex. It should be noted that the above method of testing four out of the eight classes will detect all crossovers between *a* and *T(5;8)a* only if *a* and *pa* lie on the same side of the break. If they lie on

opposite sides of the break, and if crossovers can occur simultaneously between both loci and the break, some, although presumably a minority, of the crossovers may have the phenotypes *pa a*+ and ++*b* and hence go undetected.

Additional data on the linkage of *a* and the break came from two matings, *pa a T(5;8)a B/pa A*+*B*×*pa A*+*B/pa A*+*B* and *Pa a T(5;8)a b/Pa A*+*b* ×*Pa A*+*b/Pa A*+*b*, designed primarily not to provide linkage data, but to provide mice for a cross to be described in a later section. The offspring were tested for the presence of *a* and, as a by-product of this and of other matings in which some of them were used, for semi-sterility. Thirteen tested offspring where the female was the heterozygous sex yielded one probable crossover; 139 tested offspring where the male was the heterozygous sex yielded two

TABLE 3

Segregants from the mating ♀ *T(5;8)a*++/+*m b*×♂+*m b*/+*m b.**

++	*m b*	*T*+*b*	+*m*+	*T m*+	++*b*	?+*b*	?*m*+	TOTAL
85	87	4	6	0	1	1	1	185

* Only crossovers between *m* and *b* were tested for semi-sterility. The four tested mice classed as semi-sterile gave litters as follows: 3.5 (four litters), 4.5 (three), 4.8 (five), 5.3 (three). The corresponding figures for the seven normals: 5.7 (six), 6.5 (two), 6.6 (five), 7.5 (four), 8.0 (four), 8.3 (seven), 9.0 (three). One crossover was killed untested, one was sterile.

probable crossovers. Two of the three probable crossovers were further checked (and confirmed) by a test of their progeny. As previously noted, there were no crossovers in 28 mice in the original linkage test.

The totals for crossovers between *a* and the break are as follows: one in 124, or 0.8 percent, where the female was the heterozygous parent; four in 258, or 1.5 percent, where the male was the heterozygous parent. Four of these crossovers were checked by testing of progeny; one being unchecked is slightly open to question.

One more probable crossover, which may have occurred in either sex, was found among five tested *aa* mice from the cross of semi-sterile×semi-sterile discussed in a later section.

None of the crossovers serve to prove whether the break is between *pa* and *a*, or on the opposite side of *a* from *pa*. However, since on the latter assumption ♂ No. To1255 (see above) would be produced by a single crossover, whereas on the former two crossovers on adjacent arms would be required, there is perhaps a slight presumption in favor of the latter alternative.

The relation of misty (*m*), an eighth chromosome gene, to *b* and the break was determined by the two crosses listed in tables 3 and 4. These tables are self-explanatory. They show *m* to lie between *b* and the break, with 8.0±1.9 percent crossing over between *m* and *b* in the female (normal value 7.1 percent, WOOLLEY 1945), and 21.4±7.7 percent crossing over between *a* and *m*. The figures for the male are 6.7±6.3 percent and 20.3±10.4 percent. One double crossover was found.

TABLE 4

Segregants from the mating a T(5;8)a++/++m b×a+m b/a+m b.

HETEROZYGOUS SEX	a + +	+ m b	+ + +	a m b	+ m +	a + b	a m +	+ + b	TOTAL
Female	10	8	5	1	1	3	0	0	28
Male	7	4	2	1	0	1	0	0	15

The results of linkage tests with *T(5;8)a* are summarized in table 5.

TABLE 5

Crossing over in chromosomes 5 and 8 in mice heterozygous for T(5;8)a.

HETEROZYGOUS SEX	CROSSOVER PERCENTAGE BETWEEN				
	pa AND *a*	*a* AND BREAK	*a* AND *m*	*m* AND *b*	*a* AND *b**
Female	19.8±3.7%	0.8±0.8%	21.4±7.7%	8.0±1.9%	31.0±4.3%
Female (normal)	21.2±2.7%			7.1±1.1%	
Male	15.8±3.3%	1.5±0.8%	20.3±10.4%	6.7±6.3%	25.3±3.6%
Male (normal)	19.6±2.6%			8.2±3.5%	

* This column is calculated from data from crosses in which *m* was not included. In the crosses in which *T(5;8)a*, *a*, *m* and *b*, or *T(5;8)a*, *m* and *b*, were all included, there was one double crossover in 226 mice.

The backcrosses made with *T(1;?)c* showed 23.1 percent crossing over between *p* and semi-sterility in a total of 13 mice (table 1). Additional data not summarized showed a very close linkage of *c* and semi-sterility. To analyze these linkage relations in detail, the following cross was made:

$$Sh\text{-}1\ c\ T(1;?)c\ P/sh\text{-}1\ c^{ch}+p \times sh\text{-}1\ c^{ch}+p/sh\text{-}1\ c^{ch}+p$$

Animals from this mating showing a crossover between *Sh-1* and *c* or between *c* and *P* were saved for a test as to semi-sterility. Of six animals involving a crossover in the first named segment, tests were completed on all six. Of 56 animals involving a crossover in the second named segment, tests were completed on 45. In no case did semi-sterility separate from *c*. It may be added that in several other fairly extensive crosses, not designed primarily for

TABLE 6

Segregants from the mating + c T(1;?)c+/sh-1 c^ch+p×sh-1 c^ch p/sh-1 c^ch p.

HETEROZYGOUS SEX	+ c +	*sh-1 c^ch p*	+ c p	*sh-1 c^ch* +	*sh-1 c* +	+ *c^ch p*	TOTAL
Female	151	150	16	25	3	2	347
Male	46	31	8	7	1	0	93

linkage purposes, *c* has always remained associated with semi-sterility. The results of the test cross described above are summarized in tables 6 and 7, which are self-explanatory.

TABLE 7

Crossing over in chromosome 1 in mice heterozygous for T(1;?)c.

HETEROZYGOUS SEX	*p* AND *c*	DIF.	P	*c* AND BREAK	*c* AND *sh-1*	DIF.	P	TOTAL NUMBER OF MICE
Female	11.8±2.0%			0%	1.4±1.1%			347
		−4.3	.033			−2.71	.017	
Female (normal)*	16.10±0.503%				4.11±0.291%			
Male	16.1±3.4%			0%	1.1±1.8%			93
		+4.2	.22			−1.94	.30	
Male (normal)*	11.90±0.608%				3.04±0.499%			

* Combined totals from GRÜNEBERG (1943).

No tests in addition to the backcross have been made with *T(2;?)d*.

The results of the linkage studies may be summarized as follows:

The crossing over in marked chromosome segments is reduced as compared with the normal value in all cases except two. One exception is the *p*—*c* segment in the male of *T(1;?)c*, where the observed value is 16.1±3.4 percent as compared with the normal value of 11.90±0.608 percent (GRÜNEBERG 1943). The increase is not significant (P=.22). The decrease for the *c*—*sh-1* segment in the female is significant (P=.017), and probably also that for the *p*—*c* segment in the female (P=.033). The second segment showing an increased crossing over is the *m*—*b* segment in the female of *T(5;8)a*. Again the increase is not significant. It is noteworthy that this is the only segment some distance removed from the break. This suggests that the translocation does not interfere with normal pairing in this arm, but the data are insufficient to establish this point beyond question. The reduced crossing over in other segments may be due to the mechanical action of the break rather than to reduced pairing.

The existence in *T(5;8)a* of a linkage between the normally unlinked genes *a* and *b* is proof that a translocation has occurred.

SEMI-STERILE: NORMAL RATIO

The mating semi-sterile×normal, according to theory, should give a ratio of one semi-sterile to one normal (in addition to non-viable embryos). HERTWIG (1940), as stated in the introduction, found evidence for a deficiency of semi-sterile mice. Her evidence is derived from the mean-litter-size test for semi-sterility. Evidence from this source, no matter how much care is used in gathering it, is open to the objection that there is a region of overlap in the mean size of litters produced by semi-sterile and normal mice (see, for example, table 9).

TABLE 8

Ratio of semi-steriles to normals from mating T/+×+/+ as determined by linked genes.

MATING	MARKER GENES	OBSERVED RATIO	UNCLASSIFIED	PERCENTAGE SEMI-STERILE (OR CROSSOVERS)
+*a T(5;8)a*+/*pa*++*b*×*pa a*+*b*/*pa a*+*b*	*pa*:*Pa*†	140:145	3	50.9±3.0
Same	*A*:*a*	134:128	26	
a T(5;8)a/++×*a*+/*a*+	*A*:*a*	28:43	5	50.7±2.3
a T(5;8)a/++×++/++ fully tested*	*A*:*a*	74:68	159	
Same test incomplete*	*A*:*a*	4:7		
T(5;8)a+/+*m*×+*m*/+*m*	*m*:*M*	109:119	5	52.2±3.3
+*c T(1;?)c*+/*sh-1 c*^{ch}+*p*×*sh-1 c*^{ch} *p*/*sh-1 c*^{ch} *p*	*p*:*P*†	214:241	10	53.0±2.3
Same	*c*^{ch}:*c*	215:225	25	52.3±2.1
c T(1;?)c/*c*^{ch}+×*c*^{ch}+/*c*^{ch}+	*c*^{ch}:*c*	67:84	4	

† With a few exceptions, pregnant females were checked daily and newborn litters classified as to eye color.

* Offspring from this cross were phenotypically agouti and had to be classified as *AA* or *Aa* by a genetic test. The test consisted of mating to *aa* mice; five *A* young were regarded as proof that the parent was *AA*. A few mice giving four *A* young, or proven semi-sterile or normal by litter size test only, are classified as "test incomplete."

The establishment of genes linked with *T(5;8)a* and *T(1;?)c* made it possible to use genetic markers to establish the frequency of the two classes. The genes *a* and *c*, because of their close linkage with the breaks in the two translocations, are particularly suitable for this purpose. The genes *pa*, *m*, and *p* can also be used. These markers have the disadvantage, as compared with *a* and *c*, that they are farther from the breaks; the first and third have the advantage that, in the crosses used, they could be identified in new-born animals. It will be seen from table 8 that in every case there was a slight but not significant excess of the gene that entered the cross with semi-sterility. There is no evidence for a departure from the expected 1:1 ratio.

PERCENTAGE FERTILITY OF SEMI-STERILES

KOLLER (1944) has summarized much of the literature dealing with the fertility of translocation heterozygotes in different species. No further summary will be given here. Suffice it to say that in many cases, within the limits of statistical error, there is just 50 percent reduction in fertility. In some species there is much less than 50 percent reduction in fertility; in certain cases there is more than 50 percent reduction.

As the measure of fertility of translocation heterozygotes in mice we have used the "percentage fertility," defined as the mean litter size of semi-sterile mice, divided by the mean litter size of a comparable group of normal mice, times 100. The data in regard to the percentage fertility of the six translocation stocks are summarized in tables 10 and 11. The values range, for semi-

TABLE 9

Frequency distribution of backcross animals with respect to the mean sizes of their litters.

	TOTAL	1.1 1.5	1.6 2	2.1 2.5	2.6 3	3.1 3.5	3.6 4	4.1 4.5	4.6 5	5.1 5.5	5.6 6	6.1 6.5	6.6 7	7.1 7.5	7.6 8	8.1 8.5	8.6 9	9.1 9.5	9.6 10	10.1 10.5	10.6 11	11.1 11.5	11.6 12	12.1 12.5	12.6 13	13.1 13.5
T(5;8)a																										
normal	53									1	3	3	10	2	7	7	7	6	2	1						
semi-sterile	60		1	0	7	5	12	13	12	8	2															
T-F₁111																										
normal	43										1	3	3	6	7	6	9	3	1	2		1		1		
semi-sterile	54		2	7	11	16	9	3	4	0	2															
T-F₁194																										
normal	35								2	4	0	5	5	5	6	2	1	2					1		1	1
semi-sterile	40			5	11	10	10	2	1	1																
T-R₁8																										
normal	37										2	5	6	4	5	5	2	5	3							
semi-sterile	43			1	3	2	6	4	8	11	8															
T(1;f)c																										
normal	43										2	3	4	8	9	5	2	5	3	2						
semi-sterile	40	1	2	4	11	4	6	7	2	3																
T(2;f)d																										
normal	40										1	2	5	9	6	7	4	1	2	2			1			
semi-sterile	34		1	1	6	7	12	2	2	3																

TABLE 10

Percentage fertility of semi-sterile mice from six translocation stocks.

CROSS NUMBER*	TRANSLOCATION	FEMALES					MALES				
		NORMAL		SEMI-STERILE		PERCENTAGE FERTILITY**	NORMAL		SEMI-STERILE		PERCENTAGE FERTILITY**
		NO. OF LITTERS	MEAN LITTER SIZE	NO. OF LITTERS	MEAN LITTER SIZE		NO. OF LITTERS	MEAN LITTER SIZE	NO. OF LITTERS	MEAN LITTER SIZE	
1	*T-F*$_1$*194*	30	9.37±.53	79	3.61±.19	38.4±2.9	125	7.22±.20	126	3.04±.13	41.3±1.8
1	*T-F*$_1$*111*	26	10.11±.30	42	4.33±.31	42.9±3.3	77	7.51±.25	108	3.31±.14	45.0±1.9
1	*T(2;?)d*	131	8.66±.19	100	4.25±.16	49.1±2.1	176	7.48±.17	93	3.48±.18	47.3±2.5
1	*T(1;?)c*	102	7.84±.21	82	3.51±.17	44.7±2.6	220	7.29±.15	117	3.88±.17	52.7±2.4
2		65	7.74±.28	187	3.52±.11	45.4±2.2	108	7.24±.22	127	3.84±.15	53.0±2.6
1	*T(5;8)a*	174	8.34±.15	168	4.20±.14	50.4±1.9	190	7.39±.17	253	4.55±.12	61.8±1.7
3		50	7.20±.29	46	3.33±.23	46.2±3.7	82	7.83±.43	56	4.68±.44	59.8±6.5
4		89	6.69±.19	85	3.38±.15	50.5±2.7	32	6.56±.35	104	3.90±.14	59.5±3.8
1	*T-R*$_1$*8*	59	8.44±.25	61	5.25±.21	62.2±3.1	185	7.25±.17	230	5.07±.13	68.9±1.9
	total (cross 1)						973	7.36±.07			

* Cross 1 is the group of linkage crosses listed in table 1 and other similar crosses not listed, with the exception that females from the backcrosses to the WA-stock and the V-stock are not included because many of them were homozygous for detrimental recessives tending to cut down litter size. Under cross 2 are listed litters by tested offspring from the mating $+c\ T(1;?)a+/sh\text{-}1\ c^{ch}+p \times sh\text{-}1\ c^{ch}+p/sh\text{-}1\ c^{ch}+p$. Under cross 3 are listed litters by tested offspring from the mating $+a\ T(5;8)a+/pa++b \times pa\ a+b/pa\ a+b$. For these three crosses, all males were tested by matings to B alb C females. Note the resulting uniform mean litter size for the normal males. Cross 4 was the test for the presence of *A* or *a* in young from the matings $+a\ T(5;8)a\ b/+++b \times +++b/+++b$ and $pa\ a\ T(5;8)a+/pa+++ \times pa+++/pa+++$. Lack of hybrid vigor in the females and the large proportion of first litters accounts for the small litter size for females. Males were tested by mating to stocks other than the B alb C, mostly of lower fertility. In cross 1, the identification of mice as semi-sterile or normal was based solely on the mean size of their litters; in crosses 2, 3, and 4 it was possible to check the identification on the basis of litter size by means of a very closely linked gene.

** The standard error of the percentage fertility is calculated from the formula $E_{x/y} = 100\frac{\bar{X}}{\bar{Y}}\sqrt{\left(\frac{E_x}{\bar{X}}\right)^2+\left(\frac{E_y}{\bar{Y}}\right)^2}$ where $\bar{X}$ is the semi-sterile mean litter size and $\bar{Y}$ the normal mean litter size. For cross 1 males, the value used for $\bar{Y}$ is 7.36±.07, the normal mean litter size for all cross 1 males combined.

sterile females, from 38.4±2.9 percent to 62.2±3.1 percent; for males, from 41.3±1.8 percent to 68.9±1.9 percent. There is no question that these extreme values differ from 50 percent. From table 11 it will be seen that P, the probability of a deviation from 50 percent as great as or greater than that observed, occurring due to chance alone, is, for semi-sterile males, <.0001 for *T-F*$_1$*194*, .0085 for *T-F*$_1$*111*, <.0001 for *T(5;8)a* (cross 1), and <.0001 for *T-R*$_1$*8*. It can safely be concluded that for the first two of these translocations the mean size of litters produced by heterozygous males is less than 50 percent of normal, and for the last two more than 50 percent of normal.

Tables 10 and 11 reveal another interesting fact—namely, a difference in percentage fertility between sexes. In every case except one, *T(2;?)d*, the percentage is higher in the male than in the female. The difference is greatest in the case of *T(5;8)a*, being 11.4, 13.6, and 9.0 for the three crosses in which this translocation was used. The value of P for the first of the crosses is <.0001, so that in this case the difference is unquestionably significant.

That these differences in fertility between translocations and between sexes have real biological, as well as statistical, significance, is indicated by the

TABLE 11

Significance of differences in fertility between translocations and between sexes.

CROSS NUMBER*	TRANSLOCATION	PERCENTAGE FERTILITY SEMI-STERILE FEMALES	DIFFERENCE FROM 50%	P**	PERCENTAGE FERTILITY SEMI-STERILE MALES	DIFFERENCE FROM 50%	P**	DIFFERENCE BETWEEN MALES AND FEMALES	P†
1	*T-F*$_1$*194*	38.4±2.9	−11.6	.0001	41.3±1.8	− 8.7	<.0001	2.9	.39
1	*T-F*$_1$*111*	42.9±3.3	− 7.1	.032	45.0±1.9	− 5.0	.0085	2.1	>.5
1	*T(2:?)d*	49.1±2.1	− 0.9	>.5	47.3±2.5	− 2.7	.31	−1.8	>.5
1	*T(1:?)c*	44.7±2.6	− 5.3	.042	52.7±2.4	+ 2.7	.26	8.0	.023
2		45.4±2.2	− 4.6	.037	53.0±2.6	+ 3.0	.25	7.6	.025
1	*T(5:8)a*	50.4±1.9	+ 0.4	>.5	61.8±1.7	+11.8	<.0001	11.4	<.0001
3		46.2±3.7	− 3.8	.31	59.8±6.5	+ 9.8	.13	13.6	.07
4		50.5±2.7	+ 0.5	>.5	59.5±3.8	+ 9.5	.013	9.0	.06
1	*T-R*$_1$*8*	62.2±3.1	+12.2	.0002	68.9±1.9	+18.9	<.0001	6.7	.07

* See footnote table 10.

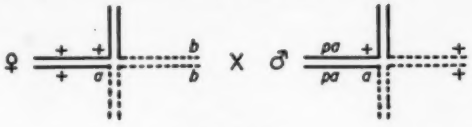
** P is derived from the Dif./SE, the Dif. being the difference from 50 per cent fertility and the S.E. the standard error of the observed percentage fertility.

† P is derived from the Dif./S.E., the Dif. being the difference in percentage fertility between males and females, and the S.E. the standard error of the difference calculated from the formula $S.E._{x-y} = \sqrt{(S.E._x)^2 + (S.E._y)^2}$.

excellent agreement between fertility percentages observed with the same translocation in different crosses. Thus in three entirely different crosses, males heterozygous for *T(5;8)a* showed percentage fertilities of 61.8±1.7, 59.8±6.5, and 59.5±3.8 (table 10).

The question next arises whether the percentage fertility coincides with the percentage of orthoploid gametes. (Orthoploid gametes are those carrying the normal haploid complement of genes, whether or not rearranged. Aneuploid gametes are those which do not contain a normal haploid complement of genes, but carry duplications and/or deficiencies of some. The first two types of sperm in figure 1 are orthoploid, the last four aneuploid.) Direct evidence on this point cannot be obtained. Some indirect evidence is furnished by an

earlier embryological study with *T(5;8)a* (SNELL, BODEMANN, and HOLLANDER 1934). Seventeen females mated to a semi-sterile male and killed at ten to 13 days after mating yielded an average of 5.22 normal embryos per female. Eleven control females yielded 8.82 normal embryos per female. The percentage fertility calculated from these data is 59.2 percent, an excellent check with the values of 61.8, 59.8, and 59.5 shown in table 10. Further embryological studies are projected, but these earlier results are sufficient to indicate that differential mortality does not materially alter the percentage fertility between 10–13 days embryo age and term.



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EGGS	pa + 1 .305	pa a + 2 .305	pa + 3 .147	+ pa a 4 .147	pa pa + a 5 .048	+ + 6 .048
+ + b 1 .250	gray .0762	gray .0762				
+ a b 2 .250	gray .0762	black .0762				
+ + b 3 .202				gray .0297		
b + a 4 .202			gray .0297			
+ + a 5 .048						gray .0023
b b 6 .048					brown pallid .0023	

FIGURE 1.—Checkerboard showing expected results from cross of *T(5;8)a* heterozygote×heterozygote. See text for explanation.

There are probably proportionately more still births, and hence more young eaten by the mother, in very small litters than in normal sized ones, but since litters were recorded soon after birth, and pains taken to locate the remains of any partly eaten young, this is probably a very minor disturbing factor.

In the next section it will be shown that the assumption that percentage fertility accurately measures the percentage of orthoploid gametes checks very well with evidence from other sources.

THE MATING SEMI-STERILE × SEMI-STERILE

Two final matings were made with *T(5;8)a* and *T(1;?)c*. These were *Pa a T(5;8)a b/Pa A+b*×*pa a T(5;8)a B/pa A+B* and *Sh-1 c T(1;?)c p/Sh-1 c^{ch}+p*×*sh-1 c T(1;?)c P/sh-1 c^{ch}+P*. The crosses were made reciprocally. The expected results are indicated in figures 1 and 2 and tables 12 and 14, and the observed results in tables 12 and 13.

The expected results for *T(5;8)a* are calculated on the basis of two hypotheses. Since the appearance of two pallid brown mice proves that the pallid and brown loci are on opposite rather than adjacent arms, this is assumed for both hypotheses.

TABLE 12

Segregants from the mating +a T(5;8)a b/+++b×*pa a T(5;8)a+/pa+++.*

FEMALE PARENT	+++	+a+	pa+b	+??	???
+a T(5;8)a b/+++b	228	62	2	174	8
pa a T(5;8)a+/pa+++	28	4	0	6	1
Totals	256	66	2	180	9
Expected, hypothesis 1	255.1	66.9	2.0		
hypothesis 2	264.3	57.7	2.0		

TABLE 13

Segregants from the mating + c T(1;?)a p/+c^{ch}+p×*sh-1 c T(1;?)c+/sh-1 c^{ch} ++.*

FEMALE PARENT	+c^{ch}c^{ch}+	+c^{ch}c+	+cc?	?c^{ch}+	?cc? (OR ??p)	???
+c T(1;?)a p/+c^{ch}+p	20	49	11	26	8	8
sh-1 c T(1;?)a +/sh-1 c^{ch}++	7	19	5	1	4	3

Hypothesis 1

The proportion of orthoploid sperm (first two types in figure 1) is taken as 0.61, the proportion of orthoploid eggs as 0.50. These figures are based on the fertility percentages shown in table 10. The proportions of the different types of aneuploid sperm assumed for hypothesis 1 are: type 3—.147, 4—.147, 5—.048, 6—.048. The corresponding figures for eggs are: 3—.202, 4—.202, 5—.048, 6—.048. These figures are selected so as to make the expected proportion of brown pallid mice agree with the observed (table 12). The agreement of these figures is thus, of course, without theoretical significance. Hy-

TABLE 14

Comparison of observed results from the mating +c T(1;?)c p/+c^{ch}+p×sh-1 c T(1;?)c+/sh-1 c^{ch}++ with results expected on various hypotheses.

	ASSUMPTIONS (SEE ALSO TEXT)								RESULTS (OBSERVED AND EXPECTED)						
	FREQUENCY OF GAMETES						% SURVIVAL *cc* COMPARED TO $c^{ch}c$								
HYPOTHESIS	1 & 2		3 & 4 (OR 5 & 6)		5 & 6 (OR 3 & 4)				*Sh-1* $c^{ch}c^{ch}$ *P*	*Sh-1* $c^{ch}c$ *P*	*Sh-1* *cc* *P*	P	*sh-1sh-1* AND *pp*	P	MEAN LITTER SIZE AT BIRTH*
	♂	♀	♂	♀	♂	♀	AT BIRTH	AT WEANING							
Observed at weaning									27	68	16		0		1.55±.13 or 2.15±.13
1	.265	.225	.235	.275	0	0	100	100	18.0	55.5	18.0		19.5	<.0001	2.84
2	.265	.225	.117	.137	.117	.137	100	100	21.8	55.5	21.8		11.8	<.0001	2.35
3	.265	.225	.117	.137	.117	.137	100	100	21.8	67.3	21.8		0		2.35
4	.265	.225	.117	.137	.117	.137	100	100	27.7	55.5	27.7	.02	0		1.84
5	.265	.225	.117	.137	.117	.137	74.0	59.0	26.9	68.4†	15.8		0		1.97
6	.265	.225	.117	.137	.117	.137	68.5	50.5	31.7	63.3	16.0		0		1.70
7	.265	.225	.235	0	0	.275	68.5	50.5	31.7	63.3	16.0		0		1.70
8	.265	.225	.235	.275	0	0	68.5	50.5	31.7	63.3	16.0		0		1.70

* The first observed mean litter size, 1.55, is that obtained when "litters of 0" (see text) are included, the second, 2.15, when "litters of 0" are excluded. The normal litter size used in calculating the expected litter size is 7.74 (table 10, cross 2).

† Of these $c^{ch}c$ mice, 14.6 percent will be exceptional in that they will be *Sh-1Sh-1* or *PP* instead of *Sh-1sh-1* or *Pp*.

pothesis 1 is the one on which figure 1 is based. The blank spaces in the checkerboard in this figure represent unbalanced and hence presumably inviable zygotes. The black (non-agouti or *aa*) mice, unless derived from gametes one of which was a crossover between *a* and the break, are homozygous for the translocation.

Hypothesis 2

The proportion of orthoploid eggs and of orthoploid sperm are both taken as 0.5, corresponding to a fertility percentage of 50 for both sexes. Otherwise the expected results are derived as in hypothesis 1.

Hypothesis 1 gives a very close fit, hypothesis 2 a somewhat poorer fit. This tends to confirm the conclusion drawn from litter size data that the higher percentage fertility of males as compared with females is a real phenomenon, and further tends to show that it is due to the production of a higher proportion of orthoploid gametes.

The proportion of gametes of types 5 and 6 assumed for hypothesis 1 is perhaps too large for the following reason. There was a high early mortality in the cross semi-sterile × semi-sterile apparently in some way correlated with difficulties in parturition due to the small litter size. The young that died during the first few days could always, and the still-born young usually, be classified by the presence of eye pigment as *Pa*. There were 174 animals so classified, and no pallid animals, among the mice that failed to reach the age (about six to ten days) when coat colors were classified. If these animals are added to the gray coated and black mice in figuring the gamete frequencies, these become for the sperm: 1—.305, 2—.305, 3—.156, 4—.156, 5—.039, 6—.039; for the eggs: 1—.25, 2—.25, 3—.211, 4—.211, 5—.039, 6—.039. This is perhaps a better estimate than that used in figure 1, but the standard error, except for gametes of types 1 and 2, would be rather high in either case.

The problem of the possible types of segregation that can be shown by the four chromosomes, or eight chromatids, which are involved in a reciprocal translocation heterozygote at first meiotic metaphase is a complex one. KOLLER (1944) reported the rather common occurrence of "numerical non-disjunction, by which three chromosomes went to one nucleus and one to the other." This would give rise to some gametes with three of the translocation chromosomes, others with only one. The higher fertility of *T(5;8)a* as compared with the interchanges studied by KOLLER, and the nearly normal crossing over, suggest that such numerical non-disjunction may be infrequent in this translocation. In any case, the union of the appropriate complementary three and one gametes to produce a viable zygote, although it can give rise theoretically, in the case of the above cross, to brown, pallid, non-agouti, non-agouti brown and non-agouti pallid mice, cannot give rise to the actually observed class, brown pallid.

KOLLER also noted that "configurations, very similar to a figure-of-eight with non-disjunctionally arranged chromosomes were often encountered." (See KOLLER's figure 4C.) If these are interpreted as involving one chiasma, or two disparate chiasmata, between a pair of centromeres and the break, and

if the non-disjunction means non-disjunction of the centromeres at the first meiotic division, then, besides gametes of classes 5 and 6 (or 3 and 4), one must infer the possibility of four new classes of gametes each carrying one of the four translocation chromosome *represented twice*, but none of the other three translocation chromosomes. The union of the appropriate complimentary gametes can then give rise to viable zygotes of the phenotypes non-agouti, non-agouti brown pallid, type, and brown pallid. The last class, though phenotypically the same as one of the classes in figure 1, is genetically different, being normally fertile and homozygous agouti instead of semi-sterile and heterozygous for non-agouti. Of the two mice in this phenotypic class, one died shortly after weaning, and the other was tested and proved to be semi-sterile and heterozygous for non-agouti.

It is worthy of note that, because of the absence of crossing over in the male Drosophila, KOLLER's "abnormal figure-of-eight" cannot occur in the male of this species, and hence the four unusual aneuploid gametes produced by this figure are not to be expected. They are a possibility in the case of the female Drosophila, but since the complementary classes of sperm could not be formed, they would necessarily go undetected. Unfortunately for genetic demonstration of their occurrence in mice, their frequency is presumably low and the chance of finding a zygote of a genotype derivable from them alone very slight indeed.

If KOLLER's abnormal figure-of-eight produces gametes 3 and 4 (fig. 1), the normal figure-of-eight produces 5 and 6, and *vice versa*. In two of his three translocations he observed both figures. The ring-of-four and chain-of-four might also produce both groups of gametes, but it would appear from KOLLER's description that they cannot actually be identified as so doing. The two types of figure-of-eight, however, are evidence that KOLLER's interchange lines B and T, like our *T(5;8)a*, produce the four classes of aneuploid gametes shown in figure 1.

In conclusion, although it would appear to be theoretically possible for as many as 26 types of gametes to be formed by a translocation heterozygote instead of merely the six represented in figure 1, and although some of these may be formed occasionally in the case of *T(5;8)a*, it seems probable that the frequency of the different types of gametes indicated for this translocation in figure 1 is a good approximation to the actual situation. In the case of less fertile translocations, such as those studied by KOLLER or as *T-$F_1$194*, there is some reason to suppose that some of the possible gametes other than those shown in figure 1 are fairly common.

If it could be shown which of the aneuploid classes of figure 1—namely, 3 and 4 or 5 and 6—resulted from centromere non-disjunction, it would become possible to infer at which ends of chromosomes 5 and 8 the centromeres are located. Thus, if classes 5 and 6 are the centromere non-disjunction classes, the centromere is located at the pallid end of chromosome 5 and the brown end of chromosome 8. There is a possibility that a cytological study can elucidate this point.

The occurrence of black (*aa*) mice in the cross discussed above proves that the translocation homozygote is viable in the case of *T(5;8)a*. This was further checked by testing five black mice from this cross for semi-sterility. Four were normally fertile as expected, one probably semi-sterile (mean size of four litters, 3.5) and hence produced by a crossover between *a* and the break. Fourteen mice from the mating translocation homozygote times normal were all semi-sterile as expected.

The mean litter size for the mating semi-sterile × semi-sterile can be calculated in two ways. The first is simply to average the observed litters. The second takes into consideration the fact that, in this cross, pregnancies in which all embryos were inviable would occur with appreciable frequency, and includes as litters of zero cases in which females were set out as pregnant but failed to give birth to a litter. There were 15 such cases as compared with 197 cases of litters of one or more. The two averages for *T(5;8)a* are 2.60±.08 and 2.41±.09. The mean litter size for normal females of cross 4, table 10 (6.69±.19), may be used as a control, but it is probably a little low because of the large proportion of first litters. Using this figure as it stands, the expected mean litter size for the mating *T(5;8)a*/+×*T(5;8)a*/+, calculated on the basis of the gamete frequencies shown in figure 1, is 2.47. The agreement with the observed litter size, either with or without litters of zero, is satisfactory.

To test the segregation of *T(1;?)c*, the mating shown in figure 2 was made. The results are summarized in table 13. It will be seen that, as in the case of *T(5;8)a*, the translocation homozygote, recognizable by its albinism, was obtained, although the fact that there were only 16 of these at weaning as compared with 27 $c^{ch}c^{ch}$ mice suggests a reduced viability. The *cc* mice bred, as expected, like normals.

While the three classes produced by the union of balanced gametes were all obtained, the two classes, *pp* and *sh-1sh-1*, recognizable as the product of the union of complementary classes of unbalanced gametes, were both lacking. This result was totally unexpected. As may be seen from figure 2, the production of gametes 3 and 4, or 5 and 6, or 3, 4, 5, and 6 by both males and females should lead to the appearance of one or both of these particular types. Possible explanations will be considered latter.

As in the case of *T(5;8)a*, mean litter size can be calculated either with or without the inclusion of "litters of zero." The frequency distribution of litters of different sizes was: 0, 29; 1, 24; 2, 29; 3, 13; 4, 6; 5, 2; 6, 1. The mean litter size including litters of 0 is 1.55±.13; excluding them, it is 2.15±.13. Some embryological data, for which the writer is indebted to MISS GLORIA BRADY, show that in this cross, litters in which all embryos degenerate are not infrequent; hence the inclusion of some litters of zero is certainly justified. Perhaps as good a guess as any of the true mean litter size would be a value midway between the two figures given above, or 1.85. It seems reasonable to suppose that this unusually small litter size is due to the same cause, whatever it may be, that leads to the absence of the *pp* and *sh-1sh-1* classes.

DISCUSSION OF RESULTS WITH $T(1;?)c$

Below, and in table 14, are presented and discussed various possible hypotheses to explain the absence of both *pp* and *sh-1sh-1* mice in the progeny

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EGGS	1 c^{ch} + .225	2 p c + .225	3 c^{ch} + .275 or .137	4 p c .275 or .137	5 p c^{ch} + c 0 or .137	6 + 0 or .137
1 p c^{ch} + .225	*Pp* $c^{ch}c^{ch}$ *Sh-1 sh-1* .0596	*Pp* $c^{ch}c$ *Sh-1 sh-1* .0596				
2 p c + .225	*Pp* $c^{ch}c$ *Sh-1sh-1* .0596	*Pp* *cc* *Sh-1sh-1* .0596				
3 p c^{ch} + + .275 or .137				*Pp* $c^{ch}c$ *Sh-1Sh-1* .0647 or .0161		
4 p c .275 or .137			*Pp* $c^{ch}c$ *sh-1sh-1* .0647 or .0161			
5 p c^{ch} + c 0 or .137						*pp* $c^{ch}c$ *Sh-1 sh-1* 0 or .0161
6 + 0 or .137					*PP* $c^{ch}c^{ch}$ *Sh-1sh-1* 0 or .0161	

FIGURE 2.—Checkerboard showing expected results from cross of $T(1;?)c$ heterozygote×heterozygote, based on two possible frequencies of gametes 3, 4, 5, and 6. See text for explanation.

of the cross shown in figure 2. In all cases it is assumed that gametes 1 and 2 are produced with a frequency of .265 in the male and .225 in the female (table 14 and fig. 2), these figures being derived from the fertility percentage (table 10).

Hypotheses 1 and 2

Assume that homozygous *pp* and *sh-1sh-1* zygotes in fact are produced and are viable but failed to appear as a matter of chance. Assume the frequency of gametes 3, 4, 5, and 6 shown in table 14. These hypotheses are untenable, the probability of the expected classes failing to appear due to chance alone being $<.0001$.

Hypothesis 3

Assume that gametes 3, 4, 5, and 6 are produced with equal frequency (.1175 in the male and .1375 in the female) and that all zygotes shown as balanced in figure 2 survive normally but for some unexplained reason produce no *pp* or *sh-1sh-1* young. This hypothesis leads to an expected litter size of 2.35, as compared with an observed litter size of $1.55 \pm .13$ or $2.15 \pm .13$. Any frequency of gametes 3, 4, 5, and 6 other than that assumed will lead to a still larger litter size. The discrepancy in litter size renders the hypothesis somewhat unlikely if not untenable. Moreover, the difficulty of imagining a mechanism which would cause the failure of *pp* and *sh-1sh-1* young to appear is against it.

Hypothesis 4

Assume that gametes 3, 4, 5, and 6 occur with equal frequency but that the balanced as well as the unbalanced zygotes produced by them are inviable. Assume normal viability for all other balanced zygotes. This hypothesis gives an expected frequency, $c^{ch}c^{ch}:c^{ch}c:cc::27.7:55.5:27.7$, whereas the observed frequency is 27:68:16. There is a significant deficit of the *cc* (translocation homozygote) class ($P=.02$). This deficit is somewhat less at birth, the *cc* class showing a higher mortality between birth and weaning than the other classes. The translocation homozygote is not infrequently non-viable in Drosophila. It seems probable, although it cannot be regarded as proved, that the *cc* type is less viable than the $c^{ch}c^{ch}$ or $c^{ch}c$ types, and in hypotheses 5 to 8 a correction is made for this as indicated in the column headed "percent survival *cc* compared to $c^{ch}c$." The remaining hypotheses, but especially hypothesis 5, will still give a fairly satisfactory agreement with observation if this correction is not made.

Hypothesis 5

Assume that gametes 3, 4, 5, and 6 are produced with equal frequency, that *cc* mice show a 74 percent survival at birth (that is, that for every 100 $c^{ch}c$ zygotes that survive to birth, 74 *cc* zygotes survive to birth) and a 59 percent survival to weaning. Assume further that in the development of one of the two parent stocks (fig. 2)—that is, while shaker-1 or pink-eye was being introduced by crossing over, a further translocation or transposition occurred such that one of the two translocation chromosomes is hyperploid and the other hypoploid as compared with the original condition of these chromosomes, and as compared with their condition in the other parent stock. The transposition must be of such a nature as not to be lost by crossing over. Then while

gametes 1 and 2 will give rise to the usual balanced classes of zygotes (fig. 2), gametes 3, 4, 5, and 6 will give rise to unbalanced zygotes only. However, the four zygotes from gametes 3, 4, 5, and 6 shown as viable in figure 2 will in two cases be hyperploid for the new transposition only, in the other two cases hypoploid for this only. Assume, finally, that the new hyperploid class is viable, the hypoploid class inviable. The expected results under this hypothesis, both as to mean litter size and frequency of the viable classes, are in satisfactory accord with the observed results.

Hypothesis 6

Same as 5 except that both the new hyperploid and new hypoploid classes are assumed inviable, and a slightly different percentage survival of the *cc* class is postulated. This also fits the facts, the agreement as to litter size being perhaps slightly better than in the case of hypothesis 5. If hypothesis 5 or 6 is correct, the repetition of the cross of figure 2 with newly derived stocks should result in the appearance of pink-eye or shaker-1 mice.

Hypothesis 7

The special feature of this hypothesis is the assumption that the male produces gametes 3 and 4 (or 5 and 6) only, whereas the female produces 5 and 6 (or 3 and 4) only. It fits the observed data, but is rendered somewhat improbable by the fact that there is, so far as the writer is aware, no precedent for the basic assumption. A possible test for it could come from a study of the embryos from the mating semi-sterile × normal. A different frequency of abnormal types from properly controlled reciprocal crosses would be evidence favorable to the hypothesis; absence of such a difference would be inconclusive.

Hypothesis 8

In the formation of gametes 1 and 2 there is complete disjunction of the translocation chromosomes at the first maturation division; in the formation of gametes 3, 4, 5, and 6 there is disjunction along one axis only of the cross-shaped figure produced by pairing, with non-disjunction along the other axis. It is a not implausible assumption, especially if the non-disjunction axis is a long one and carries the centromeres, that the postponement of the separation of this axis and the unravelling of its chiasmata till the second maturation division would result regularly in the production of chromosome breaks. If this were the case, all zygotes produced by gametes 3, 4, 5, and 6 would be unbalanced. This hypothesis is in satisfactory accord with the facts. The best chance of confirming it would appear to be through cytological observations.

There is at the present time no basis for a choice between hypotheses 5, 6, 7, and 8. The possibility, of course, exists that none of these hypotheses is the correct one.

SUMMARY

From a group of radiation-induced translocations in mice, six were selected for intensive study. These were: *T(5;8)a*, *T(1;?)c*, *T(2;?)d*, *T-F*$_1$*11*, *T-F*$_1$*194*

and *T-R18*. These were tested for linkage against the genes *a*, *b*, *Ca*, *d*, *f Fu*, *ln*, *p*, *s*, *sh-2*, *v*, *W*, *wa-1* and *wa-2*, and in the case of *T(1;?)c* against *je*, *Re* and *Sp* also. No linkages were found in the case of *T-F1111*, *T-F1194*, and *T-R18*,, and these were therefore discarded. *T(5;8)a* was found to involve chromosomes 5 and 8, the break being about one unit from *a* and 19 units from *pa* on chromosome 5, and 21 units from *m* and 29 units from *b* on chromosome 8. *T(1;?)c* was found to involve chromosome 1, the break being very close to *c*, with crossing over somewhat reduced in the *c-p* segment on one side and the *c-sh-1* segment on the other. Semi-sterility in the case of *T(2;?)d* showed linkage with *d*.

By the use of linked genes it was possible to establish accurately the ratio of semi-sterile to normal from the mating semi-sterile × normal. The expected 1:1 ratio was closely approximated.

The fertility of the different translocation heterozygotes was tested by comparing the mean litter size from the mating semi-sterile × normal with that from comparable normal × normal matings. The values, expressed as percentages, ranged from 38.4 to 62.2 for females and from 41.3 to 68.9 for males. The differences from 50 percent are unquestionably significant in the case of four of the translocations. In the case of *T(5;8)a*, the fertility percentage is about 50 in the female and 61 in the male, the results being consistent in three entirely distinct crosses and unquestionably significant. Five of the other six translocations also show greater fertility in the male.

T(5;8)a and *T(1;?)c* are viable in the homozygous condition, though there is a suggestion of a reduced viability in the case of the latter. The other translocations were not tested.

A mating of *T(5;8)a* heterozygote × heterozygote, with appropriate marker genes, showed that *pa* and *b* lie on opposite arms and that non-disjunction may occur along both axes but is most common on the axis which does not carry the marker genes. Since centromeres must lie on opposite arms, it can be stated that if the centromere lies on the pallid end of the *pa-a* chromosome, it must lie on the brown end of the *b-m* chromosome, and *vice versa*.

A mating of *T(1;?)c* heterozygote × heterozygote with appropriate marker genes failed to produce either of the possible non-disjunction classes of young. The litter size was also unexpectedly small. Possible explanations for this are discussed.

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THE MECHANISM OF NON-RANDOM SEGREGATION OF SEX CHROMOSOMES IN MALE DROSOPHILA MIRANDA*

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INTRODUCTION

MEIOTIC segregation of a specified chromosome is said to be random when the spindle pole attained and the particular non-homologous chromosomes with which the specified chromosome assorts both appear to be a matter of pure chance. Such randomness of segregation is easily understood, for it is generally a simple consequence of bivalent formation and structure. Since the coorientation of homologues in a bivalent is fixed only with respect to the longitudinal axis of the spindle, not the poles, and because the particular orientation attained by one bivalent is ordinarily independent of the orientation of any second bivalent, disjunction and the subsequent second meiotic division must result in random segregation.

Such is the case in most animals and plants, for they have only bivalents at meiosis. But some organisms possess more than two sex chromosomes (reviews in SCHRADER 1928; WHITE 1940), and in these exceptional forms a non-random segregation of the sex chromosomes regularly occurs. Elucidation of the mechanism involved in such atypical cases promises to be of considerable aid in the construction of a general theory of chromosome mechanics. It is the purpose of the present paper to give a cytological account of the mechanism of non-random segregation of the three sex chromosomes in the male of *Drosophila miranda* Dobzhansky.

Before entering upon a description of the meiotic events in *Drosophila miranda* males, it may be helpful to point out that not less than four principal and perhaps wholly discrete types of segregative behavior of compound sex chromosomes may be recognized when descriptions of cases are reviewed. First, there are those forms with a multivalent that gives a determinate orientation of its constituents (as in some male mantids, HUGHES-SCHRADER 1943; in male Humulus, KIHARA and HIRAYOSHI 1932; etc.). Second, tehre are those forms in which no physical connections appear to be formed between the segregating members. Here there is a distance conjugation, an approximation, or "touch-and-go" behavior on the part of alternative sets of sex chromosomes (as in some male heteropterous bugs, PAYNE 1909; in male Frullania, LORBEER 1934; etc.). Third, the compound sex chromosomes may all be of the same type and, in spite of the absence of a partner or partners of opposite kind, all segregate to the same pole (for example, in X^1X^2 OO males of spiders, PAINTER 1914; in the bug Syromastes, WILSON 1909; etc.). Fourth,

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a sex chromosome bivalent and a univalent, or two or more sex chromosome bivalents, although totally unconnected with one another, nevertheless segregate in such fashion that two mutually exclusive sets of sex chromosomes are regularly sorted to opposite poles of the spindle.

In all these types, so far as is known, it is a random matter to which pole of the meiotic spindle the X- or Y-complex of the compound sex chromosomes migrates. These segregations, therefore, are non-random purely with respect to the particular numbers and kinds of chromosomes separated from each other. Collectively they are in contradistinction to cases of non-random, *polarized*, segregation in which a particular chromosome migrates to a specified pole more or less frequently than would be expected on chance alone (as in maize, RHOADES 1942; in a moth, SEILER 1920; etc.).

Now an understanding of cases of the first type, in which multivalents are formed, would allow a generalization of the largest and least specialized class. The second and third classes are of considerable interest in connection with temporally displaced pairing and peculiarly specialized behavior on the part of spindles. But the most puzzling and, if real, theoretically most significant are those of the fourth type in which unconnected bivalents and chromosomes show correlative orientation and consequent non-random segregation. Unfortunately, none of the few cases inferred to be of the latter type are established beyond doubt. The sex chromosomes of *Gryllotalpa borealis* Burmeister (PAYNE 1912, 1916) have long been considered to undergo a non-random segregation of an unequal bivalent and a univalent. This they may in fact do, but a reinvestigation employing modern techniques must be carried out before the essential features involved become clear. From the account of DOBZHANSKY and PAVAN (1943) of compound, multiple sex chromosomes in *Drosophila prosaltans* Dobzhansky and Pavan, it would seem that correlative orientation of two independent bivalents may regularly occur in the male of this fly, but the essential facts are still unknown.[2]

Perhaps the most widely recognized case of such correlative orientation is that of *Drosophila miranda* as first described by DOBZHANSKY (1935). At meiosis in the male of this fly, according to DOBZHANSKY, an X^1-Y bivalent and an entirely separate, independent X^2-chromosome regularly occur. X^2 may lie anywhere on the spindle at first metaphase and early anaphase, it is said, but nevertheless it goes to the same pole as X^1 after the latter disjoins from the Y. The validity of this case was immediately challenged on theoretical grounds by DARLINGTON (1936), but DOBZHANSKY (1937) and KOLLER (1939) both reaffirmed the correctness of the original description. Nevertheless DARLINGTON's suspicions are correct, even though his own interpretation (1936, 1937) is wholly wrong, for MACKNIGHT and COOPER (1944) have shown that X^1 and X^2 both pair with Y at meiosis in the male of *Drosophila miranda*,

[2] Two principal criticisms may be made of the cytological details of DOBZHANSKY and PAVAN's account. The figures of "diakinetic" chromosomes are quite possibly drawn from some stage other than the first meiotic prophase, perhaps either somatic prophase or meiotic interphase. Furthermore, the "thinness" and "paleness" of the polytene strands may suggest male haploidy for these elements, but, as MACKNIGHT (1939) showed for the X^2 of *Drosophila miranda*, they cannot be considered conclusive evidence for such an interpretation.

and thus regularly form a trivalent. Although the work here reported was originally undertaken to elucidate what appeared to be an authentic case of correlative orientation of unassociated chromosomes, it may now be offered as a fairly detailed analysis of the mechanics of a sex chromosome trivalent in male Drosophila. As will become evident, the clarity of the case and the peculiar construction of the sex chromosomes involved make segregation in this fly of exceptional interest.

MATERIAL

Five strains of *Drosophila miranda* have been used in this study, all of which are from the stockroom of the CALIFORNIA INSTITUTE OF TECHNOLOGY. In each case the strains are descended from single females captured in nature, and derive their names from the place and order of capture. The strains used and their respective histories are briefly as follows:

(1) Olympic-1 was originally collected by DOBZHANSKY in 1934 in the Olympic Mountains near Brinnon, Washington. Olympic-1, along with Cowichan-1 and Cowichan-7, served as type material both for the description of the species and for DOBZHANSKY's (1935) account of determinate segregation of the sex chromosomes.

(2) Whitney-60 was also collected by DOBZHANSKY, having been taken in 1937 on the eastern slope of Mount Whitney, California. KOLLER (1939) presumably used this strain and Olympic-1 in his cytological studies confirming DOBZHANSKY's account of determinate segregation.[3]

(3) Big Basin-2.

(4) Big Basin-4 and the preceding were both collected by STURTEVANT in 1941 from a grove of redwoods in Big Basin State Park, north of Santa Cruz, California.

(5) Monterey was taken by DOBZHANSKY in 1940 near Carmel, Monterey Peninsula, California.

Except for the preliminary studies, flies were reared on SPASSKY's (1943) cream of wheat and molasses fly medium upon which they prospered, and cultures were continuously maintained at $19° \pm 0.3°$C. Early experiments were made with flies reared on a cracked-oat, molasses and cotton-seed food at a temperature fluctuating between 16° and 20°C.

CYTOLOGICAL METHODS

Owing to the general unsuitability of paraffin sections and aceto-carmine and aceto-orcein squash preparations for the study of spermatogenesis in Drosophila, all slides were made by fixing nearly unicellular films of the spermatocytes directly upon coverslips. The fixative which gave best results was Allen's B15 made up according to BAUER's (1931) formulas.

[3] DOBZHANSKY and KOLLER (1938) and KOLLER (1939) have described the Whitney strain as a new "race" distinct from the Olympic strains. This seems hardly justified, for the only constant differences between the Whitney and Olympic strains are said to be an inversion in the right limb of X^1, a possible size difference between the Y chromosomes which I have not been able to confirm, and slight mating preferences. Although these authors and DOBZHANSKY and EPLING (1944) seem to consider the Whitney strain as a "disjunct colony" in the Sierra Nevadas, it is by no means clear that sufficient collecting has yet been done to map out the actual distribution of *D. miranda*.

To prepare testis smears of Drosophila a last instar larva and a late male pupa of the same strain were placed on a coverslip in a moist chamber. The moist chamber was designed to allow dissecting and smearing to be carried out within it under the dissecting microscope. The pupa was removed from its puparium and pupal envelope, and the testes quickly pulled from the abdomen. The central area of the coverslip upon which the testes lay was wetted with larval blood. Thereupon the contents of the testes were rapidly smeared directly in the film of blood. The coverslip was immediately inverted onto the surface of freshly mixed B15, where it remained for one to not more than eight hours. Thereafter the smear was stained with Grübler's Gentian Violet according to SMITH's (1934) procedure, and mounted in a 60 percent solution of Clarite "X" in toluene. Successful preparations made in this way are characterized by remarkably well fixed and stained spermatocytes, even those at early diakinesis being exceptionally clear.

Nearly perfect fixation is not all that must be achieved with such difficult material; accurate microscopy is also required. Observations were carried out with Zeiss 3 mm and 2 mm N.A. 1.4 apochromatic objectives, using 20× and 15× Kompens oculars, respectively, and oil immersed N.A. 1.4 achromatic-apochromatic condenser giving a nine-tenths cone. The preparations were not counter-stained, but a combination of an Aklo #396 heat-absorbent glass slip with Wratten E22 and #61 filters gave sharp definition and good contrast. The washes and other drawings were made from camera lucida sketches which in turn were enlarged by pantograph.

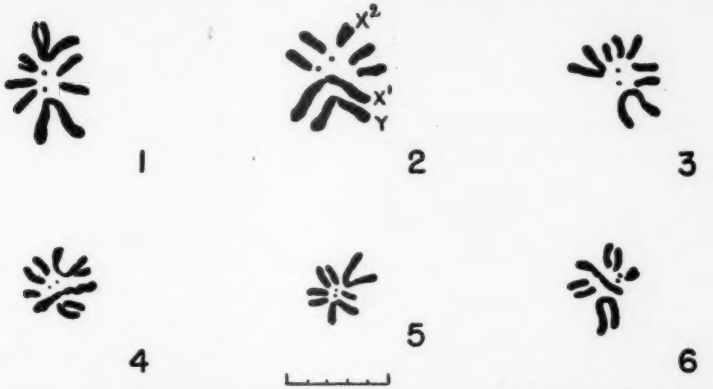
THE CHROMOSOME SET OF DROSOPHILA MIRANDA

The diploid chromosome set of female *Drosophila miranda* corresponds closely in gross morphology with the chromosome groups of race A and race B females of *D. pseudoobscura* Frolowa and Astaurow (DOBZHANSKY 1935, 1937, 1941). There are a pair of apparently equal-armed, V-shaped X chromosomes, three pairs of rod-like chromosomes (that is, chromosomes II, III, and IV), and a pair of dot-like autosomes. DOBZHANSKY and TAN (1936) have shown that each chromosome arm of *D. miranda* is at least approximately homologous (*contra* DOBZHANSKY 1941, see MACKNIGHT 1939, p. 183 *re:* the dot-like pair) to one of *D. pseudoobscura*, the principal differences between them being the result of rather extensive inversions (see MACKNIGHT 1939). This general homology is well shown by the strong somatic pairing of corresponding elements in the smaller neuroblasts of *miranda* × *pseudoobscura* hybrids (KAUFMANN 1940). The claims of DOBZHANSKY and TAN (1936) and DOBZHANSKY (1941) that more or less extensive translocations have occurred within the *miranda*-set relative to that of *D. pseudoobscura* are apparently based on incorrect identifications of chromosome parts (MACKNIGHT 1939; STURTEVANT and NOVITSKI 1941).

According to DOBZHANSKY (1935) the male of *Drosophila miranda* has one less free chromosome in its diploid set (*v.* fig. 1–6) than does the female, or male and female of *D. pseudoobscura*. There are a V-shaped chromosome corresponding with the X chromosome of the female, a somewhat unequal-

armed, V-shaped Y chromosome,[4] two pairs of rod-shaped chromosomes, an unpaired rod, and a pair of dot-like autosomes. The unpaired rod-shaped chromosome corresponds with the third chromosome of *D. pseudoobscura*. Since female *D. miranda* have two of these chromosomes, and the male apparently but one, this chromosome may be designated as a second X chromosome. Thus, if the haploid formula of the female *D. miranda* be written: X^1, X^2 (=III), II, IV, V, then the alternative haploid sets of the male gametes are: X^1, X^2, II, IV, V, and: Y, II, IV, V.

The five strains of *Drosophila miranda* here investigated have chromosome



FIGURES 1–6.—Polar views of spermatogonial metaphase plates in five strains of *Drosophila miranda*. Figure 1.—Big Basin-4. Figure 2.—Olympic, after DOBZHANSKY (1935). Figure 3.—Whitney-60. Figure 4.—Big Basin-2; right arm of uppermost sex chromosome separated into two halves. Figure 5.—Olympic-1. Figure 6.—Monterey. The scale represents five micra.

sets corresponding closely with one another and with the foregoing description (fig. 1–6). Indeed no constant difference between strains could be discerned in the chromosomes, although KOLLER (1939) states that the Y "seems larger in the Whitney race." DOBZHANSKY's (1935) belief that the spermatogonial chromosomes tend to form a specific pattern on the metaphase plate (shown in fig. 2) cannot be confirmed, as KOLLER (1939) also points out. Metaphases do occur in which X^2 lies opposite X^1 and Y on the equatorial plate as described by DOBZHANSKY, but they are by no means the rule and are never unduly frequent. Most usually X^2 lies adjacent to one of the V-shaped sex chromosomes, often the smaller of the two.

SEGREGATION IN THE MALE

Since DOBZHANSKY (1935) could find no evidence of extensive zygotic inviability, and inasmuch as X^1X^2A (A=chromosomes II+IV+V) and YA male gametes alone give normal, fertile combinations with regular X^1X^2A

[4] The *miranda* Y is incorrectly figured by WHARTON (1943, fig. on p. 307).

eggs, we appear to be confronted with a paradox. It would seem that either X^2 must segregate regularly from a chromosome with which it shares neither homology nor special attributes, namely the Y, or else X^2 must invariably assort with the equally non-homologous X^1. The cytological account by DOBZHANSKY (1935, 1937) does, in fact, portray the meiotic behavior of the sex chromosomes as anomalous in exactly this way. X^2 is said regularly to show a pattern of segregation related directly to that of the X^1-Y bivalent, although no tangible connection at any meiotic stage seems to bring about this result. Acceptance of this curious meiotic behavior at first required the analogical support of the possibly similar case of *Gryllotalpa borealis*, but KOLLER's (1939) cytological confirmation of DOBZHANSKY's description, although somewhat ambiguous, seemed to place the general account beyond doubt.

TABLE 1

The sex chromosome configurations at meiosis in male Drosophila miranda in 2035 decipherable nuclei from the following strains: Olympic-1, Whitney-60, Big-Basin-2, Big-Basin-4.

CONFIGURATION OF SEX CHROMOSOMES AT M 1	DIAKINESIS	PROMETAPHASE TO EARLIEST ANAPHASE	TOTALS
X^1YX^2 trivalent completely decipherable	110	697	807
Trivalent not completely analyzable, but no free X^2	53	1,085	1,138
X^1Y bivalent, but X^2 possibly free	8	63	71
X^1Y bivalent, X^2 definitely univalent and free	0	19	19
Total	171	1,864	2,035
Unclassifiable, all chromosomes badly overlapping or matted together	22	471	493
Grand Total	193	2,335	2,528

Be that as it may, the mechanics of segregation became openly suspect when MACKNIGHT (1939) discovered that DOBZHANSKY and TAN (1936), relying upon paleness and thinness of polytene strands as evidence of their initial haploidy, had completely overlooked the actual homolog of X^2 in the male. MACKNIGHT clearly demonstrated that most, if not all, of the euchromatin of X^2 is intercalated piecemeal into the substance of the Y chromosome, and hence the *miranda* male is not haploid for the third chromosome as was earlier believed. Now, since Y and X^1 possess a pairing segment in common (DOBZHANSKY 1935), and since Y and X^2 are partially homologous (MACKNIGHT 1939), it follows that Y may be expected to pair with both X^1 and X^2 simultaneously, forming a trivalent. This is in fact what happens at the first meiotic division (table 1), and the occurrence of such trivalents was briefly reported by MACKNIGHT and COOPER (1944).

The data of table 1 were collected by counting every nucleus in the fields analyzed. The fields chosen for analysis were selected purely on the basis of

their excellence of fixation and sharpness of staining. Of the 2035 decipherable nuclei, no more than 90 cases in which X^2 might be unpaired were found, and of these only 19 were without question cases of a univalent X^2. Since there is no reason to suppose that any different proportion of free X^2 chromosomes or of trivalents would be found among the 493 unclassifiable nuclei, it is concluded that X^2 succeeds in entering a trivalent formation with X^1 and Y in not less than 95 percent of the cases, more probably in 99 percent of all meioses. The details of the sex chromosome trivalent and its behavior will be described in detail in the succeeding cytological account.

It may be wondered how it is that DOBZHANSKY and KOLLER both misinterpreted meiosis in *Drosophila miranda* males in the same way. Possibly their belief that X^2 lacked a homolog in the diploid set of the male unconsciously led to their interpretation of early anaphase figures as those of a still earlier stage. Not infrequently (i.e., ca 10 percent of the time) X^2 may be the first element to undergo disjunction from the trivalent at anaphase. Be that as it may, it is certain from both figures and description, that KOLLER (1939) did see at least occasional sex chromosome trivalents. But these he minimized *because* they appeared in his hybrids of Olympic × Whitney strains. KOLLER further remarks that "Instances when X^2 lies close to the XY complex are none too rare in the spermatocytes of pure races."

THE FIRST MEIOTIC MITOSIS

The first meiotic stage at which nuclear details may be analyzed with confidence in *Drosophila miranda* corresponds with late diplotene or early diakinesis in other organisms. At such an early stage (fig. 7–8) the nucleus contains two relatively compact clumps, each of which proves to be a bivalent formed by one of the two large pairs of autosomes. Rarely the tiny bivalent composed of the dot-like autosomes is visible. In addition to these three there is also present a much larger element having five (rarely six) more or less contorted and projecting arms. This last is a sex chromosome trivalent, as no other body occurs in the nucleus at this time or appears later. The bivalents and trivalent stain very weakly with Gentian Violet during this period, but in the more darkly stained nuclei four chromatids may be discerned in each large autosomal bivalent, and the actual pattern of conjunction between the two X chromosomes and the Y chromosome is resolvable (fig. 8).

At this early stage the Y chromosome generally appears as a somewhat flexed, V-shaped element which seems more heavily condensed, or more compact, than the other sex chromosomes. X^1 is slender, has loose gyres in each arm, and submedially is paired with Y at what appears to be the juncture of the latter's arms. X^2 is always totally independent of X^1 and conjoined with the distal half of one arm (always the same arm?) of the Y chromosome. Not all of X^2 appears to synapse with this arm of Y, for at the earliest recognizable stages and thereafter the proximal half of X^2 is separate from it (compare fig. 8 and 9).

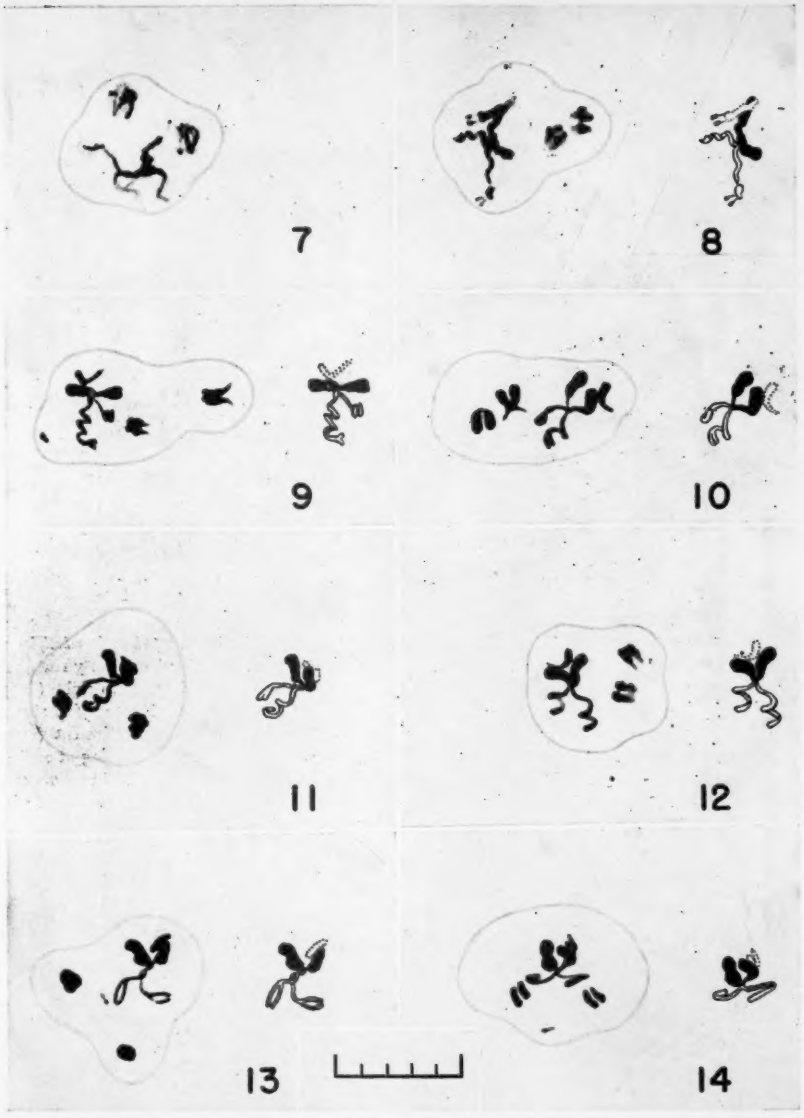
From mid-diakinesis on, the trivalent fixes and stains with such excellence that no doubt can be fostered concerning its principal details. X^1 proves to be conjoined to Y close to the kinetochore regions of both chromosomes, es-

pecially to that of the Y chromosome. It could not be determined whether X^1 has pairing segments homologous to Y in only one or both of its arms. Nor could it be determined whether Y has two such regions homologous to X^1. The fact that figures may be found in which it appears that X^1 and X^2 have both paired with the same arm of Y (fig. 21, 32), or with different arms (fig. 15g, 35), shows that Y may be amphivalent with respect to at least one of the X chromosomes. But, as would be expected with such small chromosomes, figures are very rare in which the exact locus of conjunction of X^1 and Y, and the site of Y's kinetochore, can both be determined without much doubt. Of 20 such, 15 have X^1 and X^2 definitely conjoined on different sides of Y's kinetochore, while five appear to have X^1 and X^2 conjoined on the same side of Y's kinetochore.

The most surprising feature about X^1 is that it possesses very striking relic coils in both its arms (fig. 8–12, 15a, 15b, 27) until late diakinesis, in spite of the fact that such coils are only rarely, if ever, detectable (fig. 27) at this time in the Y, and have never been discerned in X^2. As with relic coils generally, these gyres loosen and their pitch increases as prophase advances until, finally, the X^1 chromosome can no longer be said to show any vestige of the coiling of the last gonial mitosis.

As in the case of X^1, X^2 likewise proves generally to be conjoined interstitially with Y during diakinesis, for distally and proximally to the region of conjunction it may flare away from the body of the arm of Y to which it is joined (fig. 9–10, 12). Although this appears to be the rule, and many prometaphase (fig. 19–20), metaphase (fig. 21, 30, 33), and anaphase (fig. 25) figures show that such a disposition may persist until disjunction of X^2 from Y, in some trivalents the whole distal half or third of X^2 may appear conjoined with Y (fig. 11, 14, 18, 28). Whether the former configuration is a consequence of pairing with one arm of Y, the latter a result of pairing with the other arm of Y, can only be conjectured. Rare pairing conformations will be mentioned below in relation to their possible rôles in bringing about primary non-disjunction. Finally, in individual nuclei one or more of the sex chromosomes can be seen to be split in prophase (fig. 8–10, 13), so that each member of the trivalent at all of the stages described is composed of at least two chromatids.

The end of diakinesis and the onset of prometaphase is heralded by the disappearance of relic coiling in X^1, breakdown of the nuclear membrane, and consequent separation of the kinetochores of conjoined chromosomes as they coorient on the spindle (fig. 13, 14, 16, 17, 19, 20, 22, 28). A metaphase plate stage is generally formed (fig. 18, 21, 24, 29, 30, 32, 33). At metaphase all cooriented kinetochores appear to be in a state of active repulsion, frequently drawing into slender threads the proximal parts of their chromosomes between the insertions of the spindle fibers and the regions of conjunction in both autosomes and sex chromosomes alike (fig. 15d, 18, 21, 29, 30, etc.). Aside from the dot-like pair which may undergo disjunction as early as prometaphase (fig. 28) and, when visible, is generally found to be disjoined at metaphase (fig. 24, 29), anaphase comes simultaneously for the autosomal bivalents and the sex chromosome trivalent (fig. 15f, 15f′, 15g, 23, 36, 37, 39, 40). Occasionally large autosomal bivalents appear to enter anaphase before the sex



FIGURES 7–14.—Late diplotene to late diakinesis of the first spermatocyte division in *Drosophila miranda*. The diagrams to the right of the figures serve as keys to the elements comprising the sex chromosome trivalent. X^1 is represented by a line figure, X^2 by a dotted outline, and Y by solid black. X^1 is below in each case. Figure 7.—Late diplotene. Figure 8.—Early diakinesis. Figures 9–14.—Mid through late diakinesis. Figures 7–8.—Big Basin-2. Figures 9–14.—Whitney-60. Note that figure 12 is the same as photomicrograph 15b. The scale represents five micra.

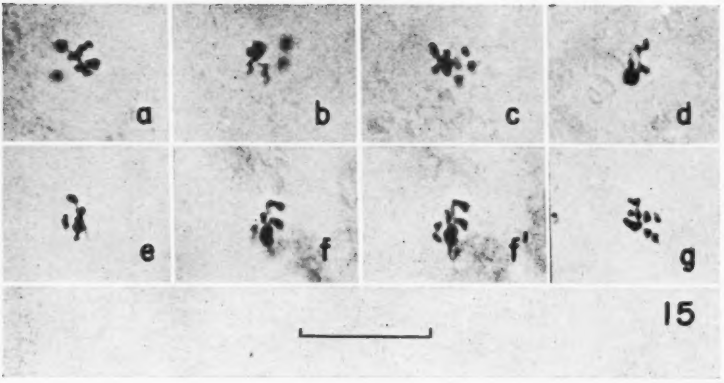
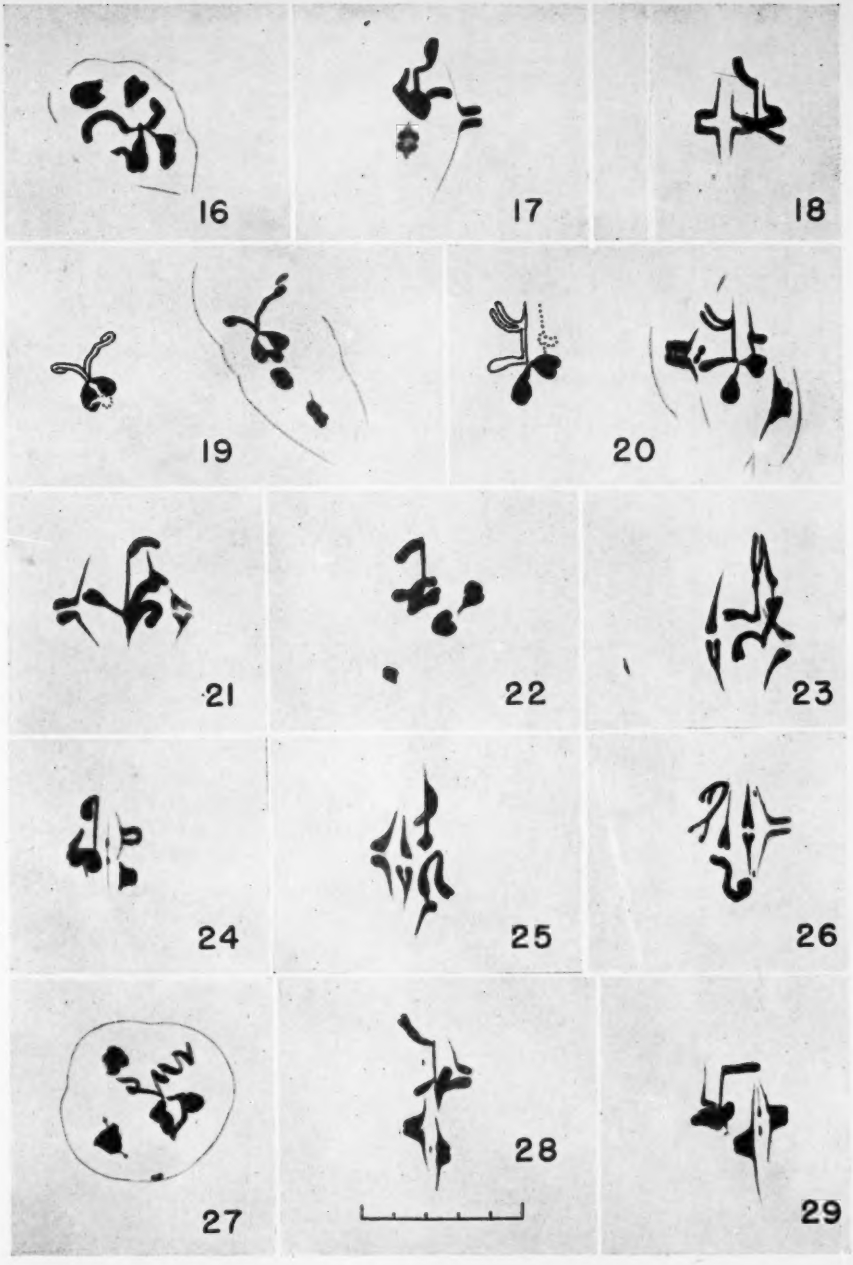
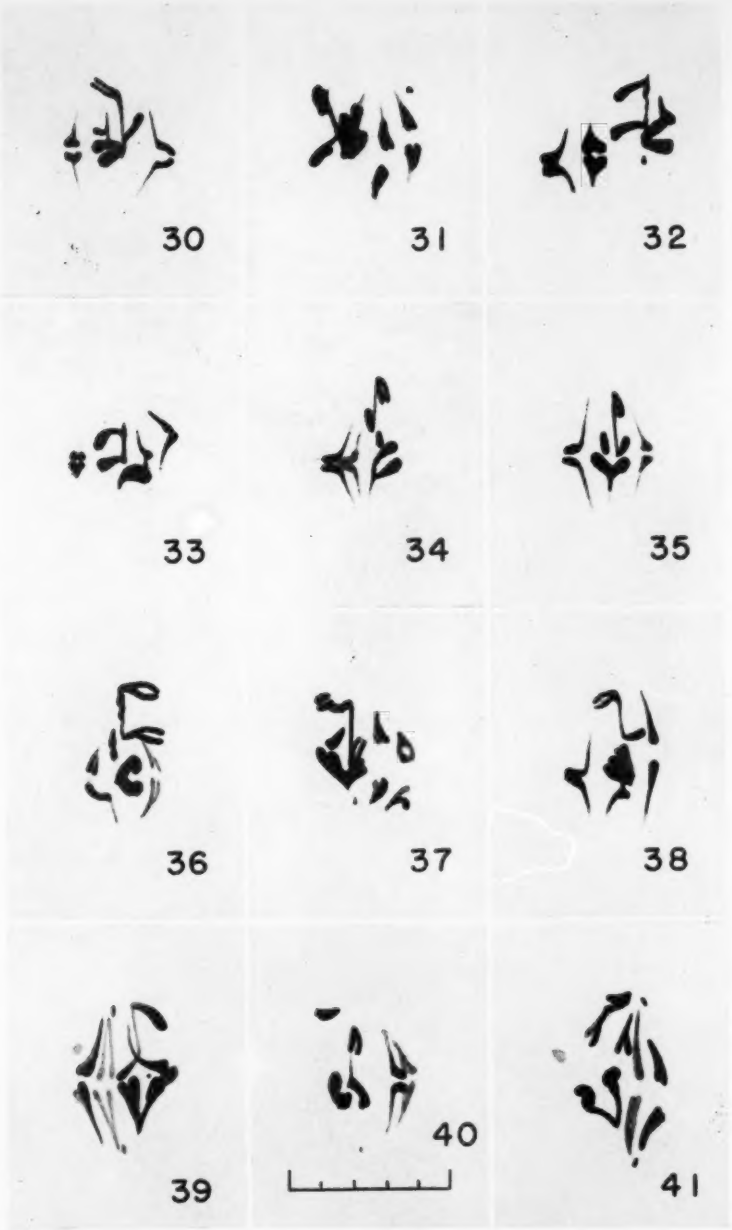


FIGURE 15.—Photomicrographs of the first spermatocyte division in *Drosophila miranda*. Figure a.—Mid-diakinesis (= fig. 27). Figure b.—Mid-diakinesis (= fig. 12). Figure c.—Early anaphase showing the sex chromosome trivalent to left and dividing autosomal bivalents to right (= fig. 31). Figure d.—Sex chromosome trivalent at metaphase; X^2 (to left) and X^1 (to right) above, Y below. Figure e.—Early anaphase, showing a misoriented sex chromosome trivalent (= fig. 38). Figures f-f'.—High and low focal depths of sex chromosome trivalent dividing at earliest anaphase (= fig. 36). Figure g.—Early anaphase (= fig. 37); X^2 is to the extreme left below one arm of X^1 and above Y. The two pairs of large autosomes are to the right. Figures a, d.—Big Basin-4. Remaining figures are Whitney-60. The scale represents ten micra.



FIGURES 16–29.—Diakinesis through early anaphase of the first spermatocyte division in *Drosophila miranda*. In all cases X^1 is toward the top of the page, as is X^2 in most of the figures; Y is generally below. Figures 16–17, 19–20, 22, 28.—Prometaphase. Figures 18, 21, 24 and 29.—Metaphase. Figures 23, 25–26.—Anaphase. Figure 27.—Mid-diakinesis (= 15a). Figures 22 and 25 are of special interest as they show a univalent X^2 and a misorientated trivalent, respectively. Figures 16–18, 22, 25.—Olympic-1. Figures 19–20.—Whitney-60. Figures 21, 23.—Big Basin-2. Figures 24, 26.—Monterey. Figures 27–29.—Big Basin-4. The scale represents five micra.



FIGURES 30–41.—Metaphase through anaphase in the first spermatocyte division of *Drosophila miranda*, strain Whitney-60. In every case, except figure 38, X^1 and X^2 are above, Y below. Figures 30, 32–33.—Metaphase. Figures 31, 34–41.—Anaphase. Figure 37 (=15g). Figure 38 (=15e) represents a misorientated sex chromosome trivalent. Figure 40 shows a univalent X^2. Figure 31 (=15c). Figure 36 (=15f, 15f'). The scale represents five micra.

chromosome trivalent (fig. 15c, 31), but equally infrequently the sex chromosomes may undergo a precocious disjunction (fig. 26, 33–35).

The actual course of anaphase presents no exceptional features (fig. 15c, 15e, 15g, 26, 31, 37, 38, 41). Even if some of the chromosomes have disjoined precociously, generally by late anaphase all chromosomes going to the same pole have their kinetochores equidistant from the spindle apex. X^2 segregates from Y just as does X^1, and accordingly X^2 is found at a pole with X^1, not at the equator, at the close of the first meiotic anaphase. One last detail from anaphase should be mentioned. At mid and late anaphase Y is often as long or considerably longer than X^1 (fig. 23, 25, 26, 34, 39, 41) when the abnormal stretching of the region of X^1 between its kinetochore and conjunctive locus is taken into account. At diakinesis, metaphase and early anaphase Y is definitely shorter and more "thick-set" than X^1. Whether, as DOBZHANSKY (1935) states, Y is actually smaller than X^1 at mitotic stages can be only conjectured from the evidence so far at hand.

The foregoing account will be found to differ radically from the earlier ones by DOBZHANSKY (1935, 1937) and KOLLER (1939). Thus it must be reemphasized that X^2 does not lie at random on the spindle nor does it lag during anaphase to become located at the equator of the spindle after all other chromosomes have migrated poleward. X^2 forms a constant member of a sex chromosome trivalent and its behavior throughout is typical of all chromosomes which regularly enter into multivalent formation and segregate nonrandomly therefrom. These results are based on the study of about five thousand nuclei and are consistently found in the spermatogenesis of *Drosophila miranda* regardless of the source of the strains which have been available for study.

THE STRUCTURE AND PAIRING OF THE SEX CHROMOSOMES

Drosophila miranda and *D. pseudoobscura* were presumably derived in relatively recent geologic times from a common ancestor. In spite of the radical innovation in *D. miranda* of a second X chromosome conjoining at meiosis with Y, conjunction of X^1 and Y apparently remains essentially the same as in *D. pseudoobscura* (compare figures in this paper with those of DOBZHANSKY 1934 and DARLINGTON 1934). This is an especially interesting circumstance because, as MACKNIGHT (1939) has shown, the Y chromosome of *D. miranda* has been very radically altered from that of *D. pseudoobscura* by having had the euchromatin of what was originally an autosome-III (element C) scattered through its substance. According to MACKNIGHT, this has presumably been brought about by the translocation of the third autosome onto Y (perhaps with the loss of one arm of Y, and in the special way that STURTEVANT and NOVITSKI (1941) conceive such translocations to occur) with subsequent repeated inversion within the III-Y fusion chromosome. The result has been a wholesale disorganization of the translocated element so that today there is but slight sequential homology between any sizeable part of Y and X^2. The differentiation between a part of the new Y and X^2 has therefore been brought about chiefly by a reorganization of Y itself. Nevertheless at least one proximal region of pairing between X^1 and Y has remained functionally unaltered

by the extensive fragmentation, inversion, and relocation that has occurred. Presumably rearrangements disrupting the conjunctive and hence segregative mechanism of X^1 and Y were quickly eliminated by strong selection against aneuploidy.

The lack of gross structural homology between X^2 and Y perhaps accounts for the fact that these chromosomes show little or no recognizable pairing tendencies in polytene nuclei. For this and other reasons, MACKNIGHT (1939) suggested that if meiotic pairing between X^2 and Y occurs, then it may involve a heterochromatic region. While such a mechanism immediately springs to mind, it must be pointed out that inferences regarding meiotic pairing properties of chromosomes have very questionable validity when deduced solely from observations on polytene chromosomes. In any event, this possibility could not be put to direct test in the present study because the slides failed to give a consistent differentiation between heterochromatin and euchromatin. The most that could be made out was that X^1 and X^2 appear never to compete for proximal (presumably heterochromatic) pairing loci in Y, nor ever to hinder one another mechanically by attempting to pair in immediately adjacent segments of Y. Rather, X^2 appeared most frequently to conjoin in the distal half of an arm of Y. Furthermore, conjunction most often seemed to involve an interstitial region in X^2 (fig. 9, 10, 12, 19, 20, 30), although configurations in which the distal length of X^2 paired with that of an arm of Y were not uncommon (fig. 8, 11, 14, 16, 18, 28).

For the most part such restricted pairing opportunities probably tend to guarantee greater regularity of determinate disjunction from a trivalent. Irregularity of meiotic behavior of homologs in trisomics and triploids forming trivalents can generally be ascribed primarily to competitive pairing (DOBZHANSKY 1933), which results in certain percentages of non-conjunction, and to non-convergent or indifferent orientations on the spindle arising very largely from the production of relatively loose, end-to-end associations[5] (DARLINGTON 1937). Both non-conjunction and non-determinate orientation lead inevitably to the occurrence of "non-disjunction" in the genetic sense, and hence to aneuploidy.

Whether the accumulation of such a succession of inversions in the III-Y fusion chromosome of *Drosophila miranda* experienced positive selection because it led to more efficient trivalent structure and segregation by eliminating competition and random orientation can only be guessed. But such a view would nicely supplement MACKNIGHT's (1939) suggestion that the III-Y fusion has selective value because it must tend to prevent the spread of "sex-ratio" (STURTEVANT and DOBZHANSKY 1936) through a population by halving the fertility of those "sex-ratio" males possessing the fusion chromosome.

[5] That loose terminal associations in a trivalent may still allow a high percentage (ca 97 percent) of determinate disjunction follows from the interesting observations of HUGHES-SCHRADER (1943) on the sex chromosome trivalent in Stagmomantis. The sort of behavior which she has described might be expected where the linearly arranged trivalent is disproportionately long relative to the effective length of the spindle. But such regularity of convergent orientation would not be expected *a priori* in forms with trivalents whose total lengths are short when compared with the spindle axis, as is the case in *Drosophila miranda*.

This would be the case because in "sex-ratio" males the Y is eliminated during the maturation divisions which, in this case, must also result in the simultaneous elimination of the attached autosome. While MACKNIGHT's hypothesis seems satisfactory to account for the persistence of the III-Y fusion chromosome once it has arisen, it nevertheless fails to explain why there apparently has also been a strong selection for cumulative loss of sequential (structural) homology between the free and Y-attached elements C. Certainly it seems hardly likely that the progressive loss of structural homology between X^2 and element C in the III-Y fusion chromosome was purely a matter of chance. Yet it is also clear that trivalent formation in *D. miranda* does not follow an absolutely stereotyped pattern, and that a more rigorous localization of conjunction between X^2 and Y might be expected to improve the overall mechanism of determinate disjunction. Although true end-to-end associations of X^2 with Y have not been observed, in a minority of the cases (less than 26 percent) X^2 may distally overlap the end of Y (fig. 21, 25, 32). Definitely in seven instances, and perhaps in as many as 26, X^2 actually paired in reversed fashion with Y so that the lengths of the respective chromosomes were directed, relatively to their kinetochores, in opposite directions (fig. 38). Both types of pairing occurred indiscriminately among strains and gave rise—perhaps being exclusive antecedents—to the small number of linearly oriented trivalents (fig. 15e, 25, 38) which result in non-disjunction of either X^1 or X^2 from Y. Such irregular types of conjunction are not unexpected if MACKNIGHT's (1939) general interpretation of the *miranda* Y is correct, although they also suggest that meiotic conjunction of X^2 and Y does not necessarily involve heterochromatic regions. Perhaps further selection for disorganization of structural homology between X^2 and the III-Y fusion chromosome may be expected to result in still greater rarity of configurations giving rise to linear orientations.

From the foregoing argument, such reorganizations of sequence would be expected to occur most frequently within Y. Surviving inversions in the free X^2 for the most part should involve only relatively short proximally or distally located segments, as is the case for the two inversions in X^2 which KOLLER (1939) has recorded.

At any rate, the *miranda* X^1YX^2 trivalent is today about 97 percent efficient, as follows from the facts that: (1) non-conjunction of X^1 and Y was not observed in a total of 2035 cells in which it would have been possible to recognize its occurrence. (2) Non-conjunction of X^2 and Y was established cytologically in only 19 of 2035 cells, and occurred in not more than 90 of 2035 meiocytes. This gives a frequency of "non-disjunction" of X^2 and Y lying somewhere between 0.4 percent and 2.2 percent. (3) Only 12 misoriented (that is, linearly oriented) trivalents were found among 1864 first meiocyte divisions in which the pattern of orientation could be made out. Thus the overall failure of determinate X^1X^2-Y segregation lies normally between 1 percent and 3 percent.[6]

[6] MACKNIGHT (1939) was able to detect genetically only one of the four types of gametes resulting from primary non-disjunction of X^1 and Y—namely, X^2A gametes. His data suggest about 1.5 percent linear orientations of the trivalent, a value approximately twice that (0.64 per

CHIASMATA AND CONJUNCTION OF THE TRIVALENT

DARLINGTON (1931, 1934, 1939) has consistently maintained that X and Y conjoin at meiosis in male Drosophila by constantly occurring reciprocal chiasmata. Were it not for the fact that this interpretation has gained a wider acceptance of DARLINGTON's primary "chiasma hypothesis of metaphase pairing" than either facts or theory merit, no further discussion than that already given (COOPER 1941, 1944a, 1944b, 1945) would be called for here. Perhaps it is of some value to point out merely that the structure and behavior of the *miranda* sex chromosome trivalent is in agreement with my earlier conclusion that the reciprocal chiasmata hypothesis is not a necessary inference from the primary data. Thus X^2 in conjoining with and disjoining from Y parallels the behavior of X^1 to a most remarkable degree when association is interstitial for both X^2 and Y (fig. 9, 10, 12, 20, 30, 35–37, 39, 41). The common mode of conjunction of X^2 with Y is therefore cytologically indistinguishable from that by which X^1 forms an association with Y. But the variable patterns of conjunction of X^2 with Y lead to no cytologically discernible differences in actual mode of association. Therefore, either Y invariably elicits reciprocal chiasmata in conjoining with X^2, as it is supposed by DARLINGTON (1936) to do in conjoining with X (X^1), regardless of site or direction of pairing, or else such a pattern of chiasmata is not essential for metaphase association. Since X^2 and Y differ structurally by a multitude of sequences, the former interpretation appears inadmissable without the introduction of a number of special subsidiary assumptions. Accordingly it may be supposed that X^1 and X^2 conjoin with Y by similar mechanisms and that the general mechanism involved is independent of crossing over—hence of chiasmata.

The above conclusion is in accord with that derived from comparative studies of the flies Melophagus (COOPER 1941), Trichobius (COOPER 1942), and Olfersia (COOPER 1942, 1944a, 1944b), as well as from a review of the relevant literature (COOPER 1944b). Since MATHER's (1944) recent genetic evidence for the regular occurrence of reciprocal chiasmata between X and Y in male *Drosophila melanogaster* is based upon faulty experiments (see COOPER 1945, pp. 479–480), there can no longer be said to exist any published data uniquely requiring such an interpretation of meiotic conjunction in male Diptera. This is not surprising, for the primary "chiasma hypothesis of metaphase pairing" itself (which alone demands the reciprocal chiasmata interpretation) has been shown not to be of general application. Thus sex chromosome bivalents devoid of chiasmata nevertheless undergo normal segregation in *female Drosophila melanogaster* (COOPER 1945). How widely the chiasma hypothesis of metaphase pairing may be applied remains to be demonstrated, because, although widely employed, it has been little subject in the past to unbiased experimental test.

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cent) for the cytological data presented here. MACKNIGHT's estimate may well be distorted by viability complications, since it is derived from the hybrid progeny of *Drosophila pseudoobscura* females by *D. miranda* males. In any event there is fair agreement between the independent genetic and cytological estimates.

SUMMARY

Contrary to the earlier descriptions by DOBZHANSKY and KOLLER, an X^1YX^2 trivalent is regularly formed at the first meiotic division in male *Drosophila miranda*.

The non-random segregation of X^1 with X^2 is explained by the fact that both these chromosomes conjoin with Y and segregate from it.

The X^1YX^2 trivalent is not less than 97 percent efficient in giving euploid gametes, and more probably is nearer 99 percent efficient.

Failures in segregation are related to non-conjunction of X^2 and Y, and to aberrant modes of association of X^2 with Y leading to linear orientations of the trivalent.

It is suggested that the loss of sequential homology between X^2 and the III-Y fusion chromosome may be accounted for by its possible rôle in the elimination of pairing competition and truly terminal associations. These two factors seem to be primarily responsible for randomness of segregation in trisomics and triploids.

X^1 and X^2 conjoin with Y by similar mechanisms not involving crossing over, hence independent of chiasmata.

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MOSAIC DOMINANCE IN THE INHERITANCE OF COLOR PATTERNS IN THE LADY-BIRD BEETLE, HARMONIA AXYRIDIS*,[1]

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INTRODUCTION

HARMONIA AXYRIDIS Pallas is a species of the coleopterous family Coccinellidae. Its distribution extends from southern Siberia (Altai Mountains) to Manchuria, Korea, Japan, and China. The color patterns on the elytra, and also on the pronotum, of this species are extraordinarily variable, so much so that some of the color patterns have been described by the old school of taxonomic entomologists as belonging not only to several species but also in fact to different genera. No less than two hundred different color forms are known from different localities (HEMMELMANN, in MADER 1932, TAN and LI 1932). As shown by DOBZHANSKY (1924) and by TAN and LI (1932), each of these color forms is found in only a part of the geographic distribution area of the species, and the population of each geographic region can be described in terms of the relative frequencies of the color patterns found in it.

In the entomological literature, the color variants of *Harmonia axyridis* have been given Latin names, subject to the taxonomic priority rules. The above quoted HEMMELMANN carried this to the extreme by giving a separate name to each variant. He is responsible for the great majority of a total of 105 names listed by KORSCHEFSKY (1932). This is, of course, a superfluous and unscientific procedure. The writer found it most convenient to classify the entire variety of color patterns in fifteen classes represented in figure 61. The genetic analysis of the color patterns was started by TAN and LI (1932, 1934), who found these patterns to be inherited in a simple Mendelian way. TAN and LI first assumed several loci to account for their inheritance, but HOSHINO (1940) and TAN (1942 and unpublished data) concluded that a series of multiple alleles of a single autosomal locus accounts for all the data available.

TAN (1942) pointed out an interesting phenomenon of "mosaicism" in the expression of the color pattern in individuals heterozygous for different alleles. The essence of this phenomenon is as follows. When two color patterns in homozygotes differ in that one shows a black pigmentation on the part of the elytron which is not black in the other, the heterozygote always has this part black. If each of two color patterns in homozygotes has black areas that are not black in the other, the heterozygote develops black pigmentation on

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[3] War-time location at Meitan, Kweichow, China.

any part of the elytron which is black in either homozygote. In other words, the heterozygotes form black pigment on any part of the elytron which is pigmented in the respective homozygotes. The present article reports further data bearing on this phenomenon of "mosaic dominance."

MATERIAL AND METHOD

The color patterns of *Harmonia axyridis* can be divided in two groups. One group, to which the name *succinea* will be applied, has yellow elytra or yellow with black spots. The position of the black spots is quite constant (fig. 10), but their number varies from zero to a maximum of 19 (on both elytra together). The odd spot lies at the scutellar angle and is shared by both elytra. The size of the spots varies, and some of them may fuse together into black bands. TAN and LI (1932) found that the variations in the number and size of the black spots on the yellow background in the form *succinea* are in part determined by the length of the pupal period. A prolongation of pupal development owing to low temperatures brings more and larger spots, while acceleration of the development leads toward reduction of the spotting. The other group of color patterns has a black background of the elytra with a varying number and form of yellow or orange spots (fig. 13–23). These patterns are *conspicua*-1 (fig. 13), *conspicua*-2 (fig. 14), *transversifascia*-3 (fig. 15), *transversifascia*-1 (fig. 16), *equicolor* (fig. 17), *spectabilis*-1 (fig. 18), *spectabilis*-2 (fig. 19), *intermedia* (fig. 20), *aulica*-2 (fig. 21), *aulica*-1 (fig. 22), and *tripunctata* (fig. 23).

The initial material used in our crosses was collected in the vicinity of the town of Meitan, province of Kweichow, southwestern China. The most common color variants in this locality are *succinea* (fig. 1–11), *conspicua*-1, and *spectabilis*-1. Less frequently occurring types are *aulica* and *transversifascia*. As indicated above, the name "*succinea*" is used to include all forms with yellow background of the elytra, regardless of the number of spots.

The following experimental procedure was adopted. Any type of color pattern the inheritance of which was to be studied was first of all outcrossed to a homozygous *succinea* line. Since all color patterns have pigmented parts of the elytra which are light in *succinea*, the heterozygous forms are invariably distinguishable from *succinea*. Among the offspring, those which were heterozygous for *succinea* and another allele were selected for further experiments. Consequently, with 12 alleles, a series of 11 different types of heterozygotes with *succinea* was established. These different types of heterozygotes were then intercrossed for the purpose of obtaining heterozygotes for different specific alleles in each combination.

The experiments were carried on in the summer months of 1943 and 1944. The technique was essentially the same as had been previously described (TAN 1933). For each type of mating, a pair of reciprocal crosses involving one female and one male were originally planned. In feeding the young larvae, special caution was taken to avoid contamination by wild larvae not infrequently found among the aphids. This is especially important in the crosses between the dominant heterozygotes and *succinea* ($S^aS^b \times ss$), where the

PLATE I, FIGURES 1–30. See reverse for description.

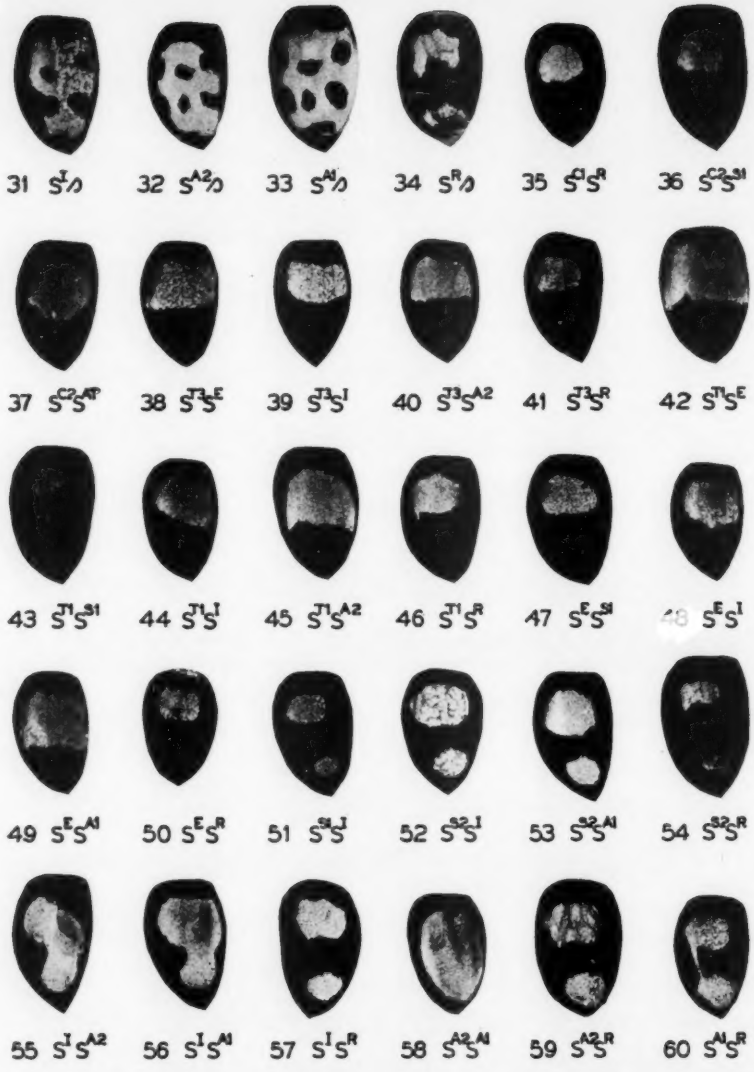

PLATE 1 and 2.—Figures 1–60: Pictures of left elytra of *Harmonia axyridis* showing the color patterns of various homozygotes and heterozygotes for 12 different alleles. The allelic symbols are: *s* for *succinea*, S^{X} for *axyridis*, S^{C1} for *conspicua*-1, S^{C2} for *conspicua*-2, S^{T3} for *transversifascia*-3, S^{T1} for *transversifascia*-1, S^{E} for *equicolor*, S^{S1} for *spectabilis*-1, S^{S2} for *spectabilis*-2, S^{I} for *intermedia*, S^{A2} for *aulica*-2, S^{A1} for *aulica*-1, and S^{R} for *tripunctata*.

presence or the absence of *succinea* in the progeny is crucial in determining the allelic behavior of these genes. Because of the frequent trouble with the aphid supply and the labor demanded in individual handling (since the larvae are carnivorous), many crosses gave finally few or even no adult offspring. This accounts for the discrepancy or the entire absence of the data in a number of cases.

All pictures were taken from the pinned material of single representative specimen for each kind brought over from China. These specimens were actually obtained from the crosses. Each picture represents only the left elytron, which was carefully detached from the beetle with the aid of a pair of fine forceps. The photographic reprints were slightly retouched in the black colored area so as to eliminate the artificial effect of light on the shining surface.

EXPERIMENTAL RESULTS AND OBSERVATIONS

Individuals heterozygous for each of the 11 types with *succinea* were obtained following the matings of the respective types to *succinea*. The genetic constitutions of these heterozygotes are: $S^{C1}s$, $S^{C2}s$, $S^{T2}s$, $S^{T1}s$, $S^{E}s$, $S^{S1}s$, $S^{S2}s$, $S^{I}s$, $S^{A2}s$, $S^{A1}s$, $S^{R}s$.

The elytral color patterns of the heterozygotes for *succinea* (*s*) and the other alleles show what could be described as a recessiveness of *succinea*, if it were not for a peculiar feature which can be denoted as presence of "indents" (see fig. 29, 30, and 34) or of small black dots within the yellow area (see fig. 26 and 28) or of both (fig. 25, 27, 31, 32, and 33). It should be recalled that the elytra of *succinea* are either yellow without spots or yellow with various numbers, up to 19, of black spots varying in size but occupying perfectly definite positions. Now, the heterozygotes between *succinea* and the other alleles invariably show black pigment on the parts of the elytra which are darkly pigmented in the homozygotes for the alleles other than *succinea*. Moreover, these heterozygotes may or may not show the "indents" or the characteristic black spots corresponding in locations to those of the spots which may or may not be present in homozygous *succinea*. Thus, heterozygotes for *conspicua*-1 and succinea ($S^{C1}s$) may either resemble *conspicua*-1 homozygotes (fig. 13) or they may be as shown in figure 24. *Conspicua*-2/*succinea* heterozygotes ($S^{C2}s$) may be as shown in figure 14 or in figure 25. *Transversifascia*-3/*succinea* ($S^{T2}s$) may be as shown in figure 15 or in figure 26. Similar comparisons can be made between figures 16 and 27, 17 and 28, 18 and 29, 19 and 30, 20 and 31, 21 and 32, 22 and 33, and 23 and 34.

It is easy to account for the origin of these variations in the forms heterozygous for *succinea*. The black spots of *succinea* appear in the heterozygotes in their proper positions, provided the developmental conditions for each spot are such as to permit their appearance. Otherwise, the spots fail to appear and the *succinea* characteristics seem completely recessive. The recessiveness is, consequently, a spurious one in this case. This interpretation has been checked by crossing individuals collected in nature and showing "indents" or spots with *succinea* from the laboratory. The individuals proved to be heterozygotes

for *succinea* and the other alleles. TAN and LI (1932) have recorded individuals with "indents" or characteristic spots in natural populations.

The results of the intercrosses of the different types of *succinea* heterozygotes are presented in tables 1 and 2 for the "inclusive" and "overlapping" types of heterozygous combinations, respectively. By inclusive type, we mean that the heterozygotes are composed of two such alleles that the black pigmented area of the pattern characteristic of one allele in homozygous condition is entirely included within that characteristic of the other allele in homozygous condition. In this case, the color pattern of the heterozygotes cannot be distinguished from that of the type of homozygote which has the more extensive black pigmented area. For example, the color pattern of the heterozygote between *transversifascia*-1 and *equicolor* ($S^{T1}S^{E}$) as shown in figure 42 is similar to that of the *transversifascia*-1 homozygotes (fig. 16). In the case of overlapping combinations, the two alleles differ so that the distribution of the black pigment characteristic of one in homozygous condition overlaps the distribution of that characteristic of the other in homozygous condition. Hence, mosaicism prevails, and the color pattern of the heterozygotes can always be distinguished from that of either parental homozygous types. The expression of the color pattern in the overlapping heterozygotes constitutes the main proof for the phenomenon of mosaic dominance.

Denoting one allele other than *succinea* as S^{a}, and another allele as S^{b}, a cross between two such *succinea* heterozygotes ($S^{a}s \times S^{b}s$) will produce $\frac{1}{4}$ so called "dominant" heterozygotes ($S^{a}S^{b}$), $\frac{1}{4}$ one parental type of *succinea* heterozygote ($S^{a}s$), $\frac{1}{4}$ the other parental type of succinea heterozygote ($S^{b}s$), and $\frac{1}{4}$ homozygous *succinea* (ss). In these tables, the two specific "dominant" alleles concerned (S^{a} and S^{b}) in each type of cross are indicated in the second column from the left. S^{a} is being referred to the allele, whose symbol is placed to the left in the column and S^{b} to the other allele whose symbol is placed to the right. In table 1, No. 1, S^{a} and S^{b} refer to the alleles S^{C1} and S^{T1} respectively, and the four classes of progeny $S^{a}S^{b}$, $S^{a}s$, $S^{b}s$, and ss are consequently represented by $S^{C1}S^{T1}$, $S^{C1}s$, $S^{T1}s$, and ss, respectively. For each cross, the genotypes of both female and male parent are given separately under the heading "parental types," and their respective origin from particular pedigree cross number is shown in parentheses. In the case quoted above (table 1, No. 1), for instance, the genotype of the female parent is $S^{C1}s$, which came from cross No. 503, while the genotype of the male parent is sS^{T1}, which came from cross No. 103. The two allelic symbols of each genotype are so represented that the one from the maternal side is placed to the left and the one from the paternal side to the right. The genotype written as $S^{C1}s$ means that the allele S^{C1} came from the grandmother and the allele s from the grandfather; the one written as sS^{T1} means that the allele s came from the grandmother and the allele S^{T1} from the grandfather. This system of reference is adopted in each of the four tables in this report. When the results of the reciprocal crosses are available, they are separately presented under one specific combination to show whether there are any differences among the reciprocal crosses. For example, in table 1, No. 7, the results for the combination $S^{T3}S^{E}$ were obtained

from two reciprocal crosses No. 143 and No. 145. If the results for one direction of mating were obtained from two separate crosses, the number of these crosses are given side by side. In table 2, No. 5, for instance, the results of the cross between $S^{T3}s$ as a female parent and sS^{E} as a male parent were obtained from two separate crosses Nos. 137 and 144.

As pointed out above, in the case of the inclusive type of heterozygous combinations, the effect on the pattern produced by one allele is covered up by that produced by the other. A distinction of "hypostatic" allele for the former

TABLE 1

Results of the intercrosses between different types of succinea heterozygotes ($S^{a}s \times S^{b}s$) for inclusive combinations.

NO.	ALLELES CONCERNED $S^{a}S^{b}$	CROSS NO.	PARENTAL TYPES (PEDIGREE NO.) ♀	♂	PROGENY CLASSES $S^{a}S^{b}+S^{a}s$	$S^{b}s$	ss	TOTAL
1	$S^{C1}S^{T1}$	180	$S^{C1}s$(503)	sS^{T1}(103)	11	4	6	21
2	$S^{C1}S^{E}$	204	$S^{C1}s$(177)	sS^{E}(124)	12	9	11	32
3	$S^{C1}S^{I}$	177	$S^{C1}s$(503)	sS^{I}(104)	15	7	5	27
4	$S^{C1}S^{R}$	228	$S^{C1}s$(180)	sS^{R}(133)	6	4	2	12
5	$S^{C2}S^{E}$	124	$S^{C2}s$(110)	sS^{E}(124)	14	9	8	31
6	$S^{T3}S^{T1}$	227	sS^{T1}(120)	sS^{T3}(122)	7	2	6	15
7	$S^{T3}S^{E}$	143	$S^{T3}s$(104)	sS^{E}(102)	(12	(9	(7	
		145	sS^{E}(102)	$S^{T3}s$(104)	17)	9)	12)	66
8	$S^{T1}S^{E}$	152	sS^{T1}(103)	sS^{E}(102)	25	12	10	47
9	$S^{I}S^{A1}$	123	$S^{A1}s$(501)	sS^{I}(104)	(15	(9	(6	
		147	$S^{I}s$(103)	$S^{A1}s$(111)	43)	24)	30)	127
10	$S^{A2}S^{A1}$	199	sS^{A2}(155)	$S^{A1}s$(123)	20	7	7	34
				Total	197	105	110	412

and "epistatic" allele for the latter is here suggested for the convenience of description. Throughout table 1, S^{a} is used in every combination to stand for the "epistatic" allele and S^{b} for the "hypostatic" allele. Since *succinea* heterozygotes for the epistatic allele ($S^{a}s$) that do not show the characteristic *succinea* effect of "indents" or spots are indistinguishable from the homozygotes for the epistatic allele ($S^{a}S^{a}$), to which the inclusive heterozygotes ($S^{a}S^{b}$) bear similarity, the class $S^{a}S^{b}$ cannot be distinguished with certainty from the class $S^{a}s$. Hence in table 1, these two classes are classified together including those $S^{a}s$ that do show the "*succinea* effect." The expected ratio should be 2 ($S^{a}S^{b}+S^{a}s$):1 $S^{b}s$:1 ss instead of 1 $S^{a}S^{b}$:1 $S^{a}s$:1 $S^{b}s$:1 ss. As the data for a number of such inclusive combinations including $S^{C1}S^{C2}$, $S^{C1}S^{S1}$, $S^{C1}S^{A2}$, $S^{C1}S^{A1}$, $S^{C2}S^{A2}$, $S^{C2}S^{A1}$, $S^{S1}S^{I}$, $S^{S1}S^{A2}$, and $S^{S1}S^{A1}$ had been previously obtained (TAN 1942), only the new combinations were experimentally tested. Data are available here for the following: $S^{C1}S^{T1}$, $S^{C1}S^{E}$, $S^{C1}S^{I}$, $S^{C1}S^{R}$, $S^{C2}S^{E}$, $S^{T3}S^{T1}$, $S^{T3}S^{E}$, $S^{T1}S^{E}$, $S^{I}S^{A1}$ and $S^{A2}S^{A1}$. As shown in table 1, the observed

TABLE 2

Results of the intercrosses between different types of succinea heterozygotes ($S^as \times S^bs$) for overlapping combinations.

NO.	ALLELES CONCERNED S^aS^b	CROSS NO.	PARENTAL TYPES (PEDIGREE NO.) ♀	♂	PROGENY CLASSES S^aS^b	S^as	S^bs	ss	TOTAL
1	$S^{T2}S^{S2}$	159	$S^{T2}s$(104)	sS^{S2}(105)	7	8	4	7	26
2	$S^{T2}S^{I}$	104	$S^{T2}s$(wild)	$S^{I}s$(wild)	(28)	(18)	(19)	(27)	
		115	sS^{I}(104)	$S^{T2}s$(104)	(25)	(19)	(23)	(16)	175
3	$S^{T2}S^{A2}$	161	$S^{A2}s$(102)	$S^{T2}s$(104)	2	3	—	—	5
4	$S^{T2}S^{A1}$	120	$S^{T2}s$(104)	$S^{A1}s$(105)	(27)	(23)	(29)	(24)	
		146	$S^{A1}s$(111)	$S^{T2}s$(104)	(4)	(8)	(8)	(13)	136
5	$S^{T2}S^{R}$	117	sS^{R}(101)	$S^{T2}s$(104)	(17)	(11)	(20)	(21)	
		137, 144	$S^{T2}s$(104)	sS^{R}(101)	(15)	(9)	(13)	(17)	123
6	$S^{T1}S^{S1}$	1005	$S^{T1}S^{T1}$(223)	$S^{S1}s$(wild)	164	175	—	2	341
7	$S^{T1}S^{I}$	103	$S^{I}s$(wild)	$S^{T1}s$(wild)	(8)	(21)	(20)	(21)	
		163	sS^{T1}(103)	$S^{I}s$(103)	(1)	(—)	(1)	(1)	73
8	$S^{T1}S^{A2}$	155	sS^{T1}(103)	$S^{A2}s$(102)	(23)	(24)	(25)	(24)	
		156	$S^{A2}s$(102)	$S^{T1}s$(103)	(2)	(12)	(4)	(5)	119
9	$S^{T1}S^{A1}$	168	$S^{A1}s$(111)	$S^{T1}s$(103)	16	15	12	10	53
10	$S^{T1}S^{R}$	154	sS^{R}(101)	sS^{T1}(103)	10	8	19	18	55
11	$S^{E}S^{S1}$	1006	$S^{E}s$(253)	$S^{S1}s$(wild)	(22)	(27)	(20)	(21)	
		1050A	$S^{S1}s$(1002)	$S^{E}s$(1020)	(7)	(8)	(5)	(7)	117
12	$S^{E}S^{I}$	126	sS^{I}(104)	sS^{E}(102)	(11)	(3)	(5)	(6)	
		149	sS^{E}(102)	$S^{I}s$(103)	(12)	(12)	(19)	(25)	93
13	$S^{E}S^{A2}$	102	$S^{A2}s$(wild)	$S^{E}s$(wild)	16	14	13	12	55
14	$S^{E}S^{A1}$	164	sS^{E}(102)	$S^{A1}s$(105)	11	6	6	11	34
15	$S^{E}S^{R}$	116	sS^{E}(102)	sS^{R}(101)	(22)	(9)	(29)	(20)	
		151	sS^{R}(101)	sS^{E}(102)	(11)	(6)	(7)	(10)	114
16	$S^{S2}S^{I}$	150	sS^{S2}(105)	$S^{I}s$(104)	13	7	16	18	54
17	$S^{S2}S^{A2}$	233	sS^{A2}(153)	$S^{S2}s$(150)	3	3	1	4	11
18	$S^{S2}S^{A1}$	105	$S^{A1}s$(wild)	$S^{S2}s$(wild)	15	5	9	17	46
19	$S^{S2}S^{R}$	232	$S^{S2}s$(209)	sS^{R}(137)	1	—	2	1	4
20	$S^{I}S^{A2}$	148	$S^{I}s$(103)	$S^{A2}s$(102)	17	15	17	16	65
21	$S^{I}S^{R}$	133	$S^{I}s$(103)	sS^{R}(101)	(7)	(8)	(9)	(6)	
		138	sS^{R}(101)	$S^{I}s$(104)	(27)	(13)	(16)	(14)	100
22	$S^{A2}S^{R}$	197	$S^{A2}s$(161)	$S^{R}s$(117)	5	—	5	2	12
23	$S^{A1}S^{R}$	166	$S^{A1}s$(111)	sS^{R}(101)	(7)	(3)	(12)	(17)	
		187	sS^{R}(133)	sS^{A1}(164)	(2)	(4)	(—)	(4)	49
			Total		558	497	388	417	1860
			Minus No. 6		164	175	—	2	341
			Total for 1:1:1:1 ratio		394	322	388	415	1519

ratios agree in most cases with the expected. Of all the possible combinations belonging to the inclusive type, experimental data are now lacking only in two cases, namely, $S^{C1}S^{T3}$ and $S^{C1}S^{S2}$. As illustrative of the nature of the inclusive heterozygotes, one finds the similarity between figure 35 ($S^{C1}S^{E}$) and figure 13 ($S^{C1}S^{C1}$), figure 38 ($S^{T3}S^{E}$) and figure 15 ($S^{T3}S^{T3}$), figure 42 ($S^{T1}S^{E}$) and figure 16 ($S^{T1}S^{T1}$), figure 51 ($S^{S1}S^{I}$) and figure 18 ($S^{S1}S^{S1}$), figure 56 ($S^{I}S^{A1}$) and figure 20 ($S^{I}S^{I}$), and figure 58 ($S^{A2}S^{A1}$) and figure 21 ($S^{A2}S^{A2}$).

The data for the overlapping dominant heterozygotes are shown in table 2. A total of 23 different combinations were recovered. These are: $S^{T3}S^{S2}$, $S^{T3}S^{I}$, $S^{T3}S^{A2}$, $S^{T3}S^{A1}$, $S^{T3}S^{R}$, $S^{T1}S^{S1}$, $S^{T1}S^{I}$, $S^{T1}S^{A2}$, $S^{T1}S^{A1}$, $S^{T1}S^{R}$, $S^{E}S^{S1}$, $S^{E}S^{I}$, $S^{E}S^{A2}$, $S^{E}S^{A1}$, $S^{E}S^{R}$, $S^{S2}S^{I}$, $S^{S2}S^{A2}$, $S^{S2}S^{A1}$, $S^{S2}S^{R}$, $S^{I}S^{A2}$, $S^{I}S^{R}$, $S^{A2}S^{R}$, and $S^{A1}S^{R}$. With one exception, all were obtained from the intercross of *succinea* heterozygotes ($S^{a}s \times S^{b}s$), and the frequencies of the four classes of individuals in the progeny fit to 1 $S^{a}S^{b}$:1 $S^{a}s$:1 $S^{b}s$:1 *ss* ratio in most cases. The observed overlapping heterozygotes, being the class $S^{a}S^{b}$, is represented in approximately 25 percent of the offspring in each type of cross. The exceptional case is shown in table 2, No. 6 representing the combination $S^{T1}S^{S1}$, which was recovered from a cross between a homozygous *transversifascia*-1 ($S^{T1}S^{T1}$) female and a *spectabilis*-1/*succinea* ($S^{S1}s$) male. As expected, this cross gives 164 *transversifascia*-1/*spectabilis*-1 overlapping heterozygotes ($S^{T1}S^{S1}$) and 175 *transversifascia*-1/*succinea* heterozygotes ($S^{T1}s$) which fit very closely to 1:1 ratio. The two *succinea* individuals maybe due to contamination or mutation or other cause. In a few cases such as for the combinations $S^{T3}S^{A2}$, $S^{S2}S^{R}$, and $S^{A2}S^{R}$ as shown in Nos. 3, 19, and 22 in table 2, the heterozygotes were recovered among a few individuals in the progeny. In several other cases, which gave only very few or no offspring, the overlapping heterozygotes were not recovered. Hence the data for combinations $S^{C2}S^{R}$, $S^{C2}S^{I}$, $S^{T3}S^{S1}$, $S^{T1}S^{S2}$, and $S^{S1}S^{R}$ are lacking.

In each of these recovered combinations, the overlapping heterozygotes can invariably be distinguished from the two parental types concerned. Some of these patterns are shown in figures 36, 39–41, 43–50, 52–55, 57, 59, and 60. These pictures cover all the overlapping types of heterozygotes recorded in table 2 with the exception of the combinations $S^{T3}S^{S2}$, $S^{T3}S^{A1}$, $S^{T1}S^{A1}$, $S^{E}S^{A2}$, and $S^{S2}S^{A2}$, for which the pictures are lacking because the elytra of these specimens were damaged beyond recovery.

By comparing the color patterns of each type of overlapping heterozygote with the color patterns of the two corresponding types of homozygotes, one is surprised to note that mosaicism prevails in all cases. As a matter of fact, the specificity of each allele is so remarkably clear cut in the heterozygote's color pattern that the relatively minor differences distinguishing between S^{C1} and S^{C2}, between S^{T1} and S^{T3}, between S^{S1} and S^{S2}, and between S^{A1} and S^{A2} can be clearly demonstrated with respect to a third allele. For example, figure 39 ($S^{T3}S^{I}$) and figure 44 ($S^{T1}S^{I}$) reveal obviously the difference between the effects of the allele S^{T3} and that of the other allele S^{T1}, since each is combined with a common third allele, which is S^{I} in this case. In other words, the difference between the characteristic patterns of any of these varieties, how-

ever little it may be, is a real one, and the autonomy of the individual effect in the developing heterozygote is true to every allele and probably in any allelic combination.

As pointed out in a preceding section, the heterozygotes between any dominant allele and *succinea* may give the characteristic "indents" or spots in the yellow area. That this manifestation is due to the effect of the *succinea* alleles alone is further substantiated by the observations that in any heterozygous combinations between two alleles other than *succinea*, the yellow area is always devoid of such modifications. Among a total of 558 overlapping heterozygotes examined (table 2) only one individual showed a small black spot in the yellow area. This was found in the heterozygote $S^{E}S^{R}$, as shown in figure 50.

Table 3 summarizes the results of the crosses between overlapping heterozygotes and *succinea*. The only inclusive type of heterozygote involved in this series of experiments was $S^{C1}S^{A2}$, which came from a cross between two homozygotes. The results show that in every successful cross, the progeny consists of two classes, $S^{a}s$ and $S^{b}s$ in an approximately 1:1 ratio. There appeared two *succinea* homozygotes, one in cross No. 122 (table 3, No. 3) between *ss* ♀ and $S^{T3}S^{I}$ ♂ and the other cross No. 191 (table 3, No. 17) between $S^{R}S^{I}$ ♀ and *ss* ♂. They were presumably due to the contamination of wild larvae in the process of breeding. Since they were found in only two individuals out of a total of 451 reported in this series of experiments, they can be considered as exceptions. The available evidence suffices to show that the various pattern forms are inherited as multiple alleles and that each of the tested dominant heterozygotes ($S^{a}S^{b}$) is composed of only two specific alleles. The allelic inheritance of various variants of color pattern types of *Harmonia axyridis* can be considered fully proven, especially because of the fact that 11 of the 12 variants are involved in this series of experiments in at least one combination (table 3) and that the relevant data for *conspicua*-2, which is not available in this experiment, was obtained in a previous report (TAN 1942).

The data from the inbreeding of the various overlapping heterozygotes and of one inclusive heterozygote ($S^{C1}S^{A2}$) are presented in table 4. The expected types and the ratio for the progeny classes should be two overlapping heterozygotes same as the parents ($S^{a}S^{b}$) to one type of homozygotes ($S^{a}S^{a}$) and to one the other type of homozygotes ($S^{b}S^{b}$). From each successful cross, the types of offspring obtained agree with this expectation. Hence, various types of dominant homozygotes are recovered. With the exception of $S^{C2}S^{C2}$, every type of dominant homozygote is represented in at least one inbred line. In fact, the pictures illustrating the color patterns of the dominant homozygotes as shown in figures 13 to 23 were taken from these individuals, the only exception being $S^{C2}S^{C2}$ (fig. 14) obtained from the previous experiments. But the numerical data concerned in this series of experiments significantly deviated from the expected 2 ($S^{a}S^{b}$):1 ($S^{a}s$):1 ($S^{b}s$) ratio in many cases. Theoretically, the proportion between heterozygotes and the sum of the two classes of homozygotes should be equal. Actually, most crosses gave more

TABLE 3

Results of the crosses between different types of overlapping heterozygotes and succinea homozygotes ($S^aS^b \times ss$).

NO.	ALLELES CONCERNED S^a S^b	CROSS NO.	PARENTAL TYPES (PEDIGREE NO.) ♀		♂		PROGENY CLASSES S^as	S^bs	ss	TOTAL
1	S^{C1} S^{A2}	1014B	ss	(1002)	$S^{A2}S^{C1}$	(1003)	(15)	(16)		
		1018A	$S^{A2}S^{C1}$	(1003)	ss	(1002)	(7)	(6)		44
2	S^{T2} S^{S2}	193	$S^{T2}S^{S2}$	(159)	ss	(wild)	4	4		8
3	S^{T2} S^{I}	122	ss	(105)	$S^{T2}S^{I}$	(104)	(33)	(31)	1	
		140	$S^{T2}S^{I}$	(104)	ss	(104)	(1)	(4)		70
4	S^{T2} S^{A1}	208	ss	(155)	$S^{A1}S^{T2}$	(146)	4	5		9
5	S^{T2} S^{R}	183	$S^{R}S^{T2}$	(117)	ss	(117)	(8)	(9)		
		184	ss	(115)	$S^{R}S^{T2}$	(117)	(9)	(6)		32
6	S^{T1} S^{S1}	1046B	ss	(1002)	$S^{T1}S^{S1}$	(1005)	7	4		11
7	S^{T1} S^{I}	139	$S^{I}S^{T1}$	(103)	ss	(101)	26	26		52
8	S^{T1} S^{A2}	221	$S^{T1}S^{A2}$	(155)	ss	(155)	4	2		6
9	S^{T1} S^{A1}	224	$S^{A1}S^{T1}$	(168)	ss	(168)	4	5		9
10	S^{E} S^{S1}	1012, 1020	$S^{E}S^{S1}$	(1006)	ss	(1002)	18	17		35
11	S^{E} S^{I}	185	$S^{I}S^{E}$	(126)	ss	(126)	8	11		19
12	S^{E} S^{A2}	132	$S^{A2}S^{E}$	(102)	ss	(102)	(2)	(2)		
		114	ss	(102)	$S^{A2}S^{E}$	(102)	(14)	(16)		34
13	S^{E} S^{A1}	241, 245	$S^{E}S^{A1}$	(164)	ss	(126)	3	5		8
14	S^{E} S^{R}	220	$S^{R}S^{E}$	(153)	ss	(155)	7	11		18
15	S^{S2} S^{A1}	170	ss	(503)	$S^{A1}S^{S2}$	(105)	8	8		16
16	S^{I} S^{A2}	194	$S^{I}S^{A2}$	(148)	ss	(138)	(6)	(7)		
		213	ss	(157)	$S^{I}S^{A2}$	(148)	(2)	(2)		17
17	S^{I} S^{R}	190	ss	(145)	$S^{R}S^{I}$	(138)	(7)	(5)		
		191	$S^{R}S^{I}$	(138)	ss	(149)	(25)	(18)	1	56
18	S^{A2} S^{R}	215	ss	(117)	$S^{R}S^{A2}$	(153)	3	2		5
19	S^{A1} S^{R}	237	$S^{R}S^{A1}$	(166)	ss	(166)	1	1		2
					Total		226	223	2	451

heterozygotes as compared to homozygotes. Some of the more significant differences are found in the inbreeding lines, $S^{T2}S^{R}$ (table 4, No. 4), $S^{E}S^{A2}$ (No. 11), and $S^{E}S^{I}$ (No. 10). In only two cases, the inbred lines of $S^{T1}S^{A1}$ (No. 8) and of $S^{T1}S^{I}$ (No. 6), the homozygotes outnumber the heterozygotes. The results are significant in showing that there is a differential survival between the homozygotes and the heterozygotes. The total number of heterozygotes is 162 and that of homozygotes is 87. The probable explanation is that some of these alleles are associated with a semi-lethal effect in homozygous condition.

TABLE 4

Results of the inbreeding of the different combinations of overlapping heterozygotes ($S^aS^b \times S^aS^b$).

NO.	ALLELES CONCERNED S^a S^b	CROSS NO.	PARENTAL TYPES (PEDIGREE NO.) ♀ AND ♂	PROGENY CLASSES S^aS^b	S^aS^a	S^bS^b	TOTAL
1*	S^{C1} S^{A2}	1014	$S^{C1}S^{A2}$ (1003)		10	5	15
2	S^{T2} S^{I}	128, 174	$S^{T2}S^{I}$ (104)	13	8	2	23
3	S^{T2} S^{A1}	205, 207	$S^{T2}S^{A1}$ (120)	6	5	1	12
4	S^{T2} S^{R}	200, 201	$S^{R}S^{T2}$ (117)	23	1	2	26
5	S^{T1} S^{S1}	1013A	$S^{T1}S^{S1}$ (1005)	10	3	4	17
6	S^{T1} S^{I}	142	$S^{I}S^{T1}$ (103)	3	6	4	13
7	S^{T1} S^{A2}	230	$S^{A2}S^{T1}$ (155)	—	1	1	2
8	S^{T1} S^{A1}	223, 243	$S^{A1}S^{T1}$ (168)	6	5	4	15
9	S^{T1} S^{R}	235	$S^{R}S^{T1}$ (154)	14	—	4	18
10	S^{E} S^{I}	195, 196	$S^{I}S^{E}$ (126)	21	—	5	26
11	S^{E} S^{A2}	171	$S^{E}S^{A2}$ (102)	19	3	8	30
12	S^{E} S^{R}	206	$S^{E}S^{R}$ (153)	3	2	—	5
13	S^{S2} S^{I}	192	$S^{S2}S^{I}$ (150)	10	5	—	15
14	S^{S2} S^{A1}	175	$S^{S2}S^{A1}$ (105)	5	—	—	5
15	S^{I} S^{R}	181, 186	$S^{I}S^{R}$ (138)	21	6	3	30
16	S^{A2} S^{R}	216	$S^{R}S^{A2}$ (153)	4	—	1	5
17	S^{A1} S^{R}	238	$S^{R}S^{A1}$ (166)	4	2	1	7
		Total for overlapping types:		162	47	40	249

* Inclusive heterozygote, S^aS^b and S^aS^a, cannot be distinguished.

DISCUSSION

Two principal conclusions emerge from the data presented above. First, the inheritance of all the types of color pattern found in our material on *Harmonia axyridis* can be accounted for by assuming 12 alleles of a single autosomal locus. Second, the color patterns to be found in the heterozygotes carrying any two alleles can be predicted on the basis of the rule of mosaic dominance. This rule states that any portion of the elytra which has black pigment in the homozygotes for a given allele will have black pigment also in the heterozygotes in which that allele is present. In other words, if the color patterns in any two different homozygotes are known, the color pattern in the heterozygote can be predicted by superimposition of the patterns of the homozygotes, and leaving unpigmented only those sections of the elytra which have no black pigment in either homozygote. A re-examination of the data of HOSHINO (1940) in the light of the rule of mosaic dominance shows that this rule is applicable also to the heterozygotes carrying the three alleles studied by him but which were not present in our material, since they are rare or absent in the population of that part of China in which our material was collected. It is very probable that the rule of mosaic dominance applies to all the 15 alleles which are known to govern the elytral color pattern in *Harmonia axyridis*.

The precision with which the rule of mosaic dominance holds is remarkable. We have traced the color patterns on the elytra of individuals homozygous for the 15 known alleles on a semi-transparent paper, and superimposed the resulting drawings in all combinations. Making black any part of the elytron which is black in either homozygote, we obtain the 105 "theoretical" patterns shown in the closed squares in figure 61. The pattern in each square shows the condition predicted in the heterozygotes carrying the alleles which, when homozygous, have the patterns represented at the top of the vertical row and at the extreme left of the horizontal row. A total of 55 heterozygotes the color patterns of which have thus been predicted were actually obtained in the experiments reported in this paper or in a previous one (TAN 1942). They are marked in figure 61 by the sign "T." Some of the heterozygotes (about 15) were obtained by HOSHINO (1940) but not in our experiments. They are marked in figure 61 by "H." Heterozygotes which have not been obtained and studied up to now are marked by a question mark in figure 61. These unknown heterozygotes are mostly combinations of the rare alleles studied by us—namely, *conspicua*-2 (S^{C2}), *transversifascia*-3 (S^{T3}), *transversifascia*-1 (S^{T1}), *equicolor* (S^{E}), *spectabilis*-2 (S^{S2}), *tripunctata* (S^{R}), and *aulica*-2 (S^{A2}), with the alleles *axyridis* (S^{X}), *transversifascia*-2 (S^{T2}) and *forficula* (S^{F}) studied by HOSHINO but not present in Chinese populations.

Comparison of the predicted patterns in figure 61 with those shown in figures 24–60 in the present paper or in papers of TAN (1942) and HOSHINO (1940) shows striking coincidence of the predicted and the observed patterns. Especially interesting, of course, are the patterns of the overlapping heterozygotes, which always have the yellow coloration restricted to only those parts of the elytra which are yellow in both corresponding homozygotes. The only variations are those produced by the "*succinea* effect" described above, which concerns the presence or absence of the black spots on the yellow background which may or may not be present in the *succinea* homozygotes. The sole exception from the rule of mosaic dominance found till now is a specimen shown in figure 50 which should have had the color pattern indicated by the sign "!" in figure 61. This specimen, presumed to be a heterozygote for the alleles S^{E} and S^{R}, should not have the black spot inside the yellow area above the middle of the length of the elytron. The presence of this spot is a characteristic of the *succinea* pattern, and the allele *s* was not supposed to be present in the specimen just referred to. The writer owes to PROFESSOR M. M. RHOADES the suggestion that this single exceptional specimen might have been a trisomic, carrying the three alleles S^{E}, S^{R}, and *s*. This appears quite probable, especially because of the fact that this specimen was obtained from a cross between $S^{E}s$ and $S^{R}s$.

On the basis of the knowledge of the inheritance of color patterns of the beetle, a re-examination of the published figures of the pattern types found in nature shows that a number of them represent, in reality, the heterozygotes of one kind or the other. The abridged forms of black varieties carrying small black spots or "indents" in the yellow area are the remarkable examples of

this kind. For this reason, the proposal of HEMMELMANN (*cf.* MADER 1932) to give a separate Latin variety name for each color pattern found in nature appears valueless. It is true that some of the published figures of HEMMELMANN or of TAN and LI (1932) do not fit into either the known homozygous or

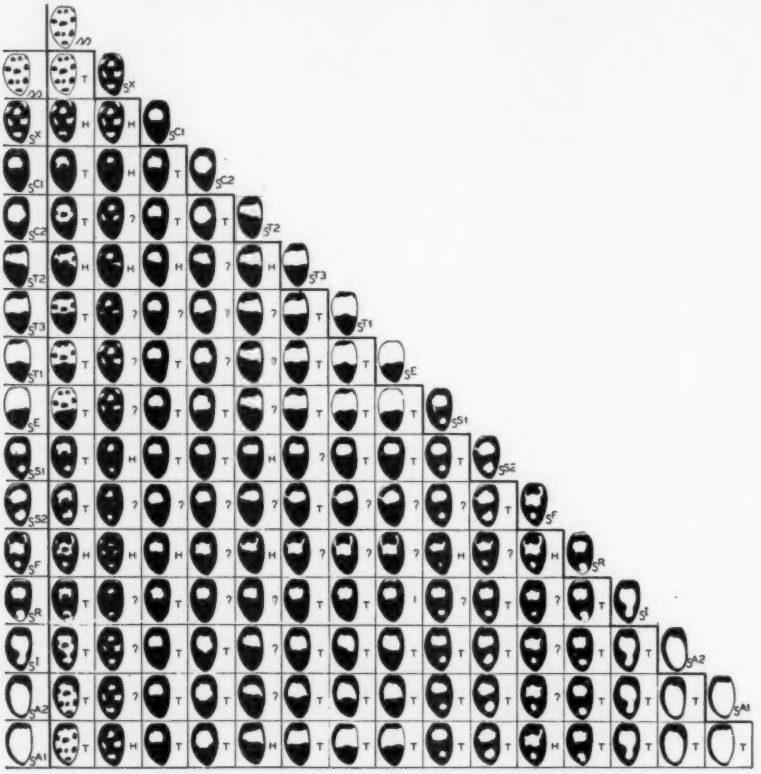

FIGURE 61.—The theoretical color patterns for the different combinations of the 15 known alleles in *Harmonia axyridis*. The symbol *T* marks the ones actually obtained by TAN in this paper or in a previous paper (1942); *H*, obtained by HOSHINO (1940);?, not obtained so far; and !, a single individual in this class showing *succinea* effect.

heterozygous combinations of the 15 alleles. One outstanding case is the pure black form known as variety *corvina* (*cf.* fig. 21 of MADER 1932). This and other cases suggest that there probably exist several more alleles which have not been included in the laboratory experiments so far.

How widespread is the phenomenon of mosaic dominance is difficult to tell. The dominance relationships in *Adalia bipunctata*, another member of the

family Coccinellidae, have been described by Lus (1928, 1932), who has also examined the inheritance of three color patterns in the related species, *Adalia decempunctata*. In neither of these species is the dominance order of the color patterns in accord with the rule of mosaic dominance. Other species of Coccinellidae have not so far been studied in enough detail to give a fair test of the applicability of this rule.

The best analogue of the behavior of the color pattern alleles in *Harmonia axyridis* is that of the scute-achaete alleles in *Drosophila melanogaster* described particularly by Serebrovsky and Dubinin (see a review in Goldschmidt 1938). The mutants at these loci are characterized by the absence of different sets of the bristles present on the body of the wild type fly. The mutants are recessive to the wild type condition, and the compounds heterozygous for any two of the mutant alleles have only those bristles missing which are missing in the homozygotes for both alleles taken separately. This so-called "step-allelomorphism" in the scute-achaete locus of *Drosophila melanogaster* has led to the so-called subgene hypothesis, which postulates that each gene is composed of a series of "subgenes," each subgene being responsible for the presence or absence of a definite bristle or a few definite bristles. The alternative explanation proposed by Goldschmidt (1931) assumes that each allele acts at a different time, and perhaps with different speed, governing the diffusion processes of some bristle-forming substances originating from some centers of the bristle pattern.

A similar hypothesis would, of course, fit the *Harmonia axyridis* also. One would have to assume that the elytron is a mosaic of many sections the coloration of each of which is determined independently from the others, or else that each allele present in an individual determines a pigment-forming "stream" in the elytron independent from the "stream" produced by the other allele. It is hardly necessary to say that there is no evidence of the existence of the pattern-forming "streams" or centers either in the scute case in *Drosophila melanogaster* or in the elytral pigmentation case of *Harmonia axyridis*. It may be noted, however, that Harmonia offers a very favorable experimental material for studies in this field. When the beetle emerges from the pupa, the entire elytron is yellowish-orange without black markings. The black pigment appears gradually, from the periphery of the elytron to its center. It has been shown by cutting parts of the elytron that some substance or substances pass from the body into the hardening elytron (unpublished data). It will be interesting to investigate further the distribution of this stream of substances in connection with the problem of the development of the black pattern.

Another analogy, possibly a more remote one, to the phenomenon of mosaic dominance in *Harmonia axyridis* is found among genes determining serological characters in the higher vertebrates. Thus, the blood group genes A and a^B in man produce the antigens A and B quite independently from each other. It is, however, rather far-fetched to suppose that each of the 15 alleles of the color pattern in *Harmonia axyridis* produces a separate substance

responsible for the deposition of the black pigment in strictly definite portions of the elytral surface.

Although some of the alleles of the elytral color pattern occur more frequently than others in the natural populations of *Harmonia axyridis*, no allele is sufficiently common throughout the distribution area to be regarded as the "wild type." The absence of a normal or wild type condition is interesting in connection with the absence in this case of the simple dominance-recessive relations usually exhibited between the wild type allele on one hand and mutant alleles on the other. Mutant alleles are presumably unfavorable for the survival of the species in all environments to which the species is normally exposed. The gene action is consequently adjusted to produce an end product which we call "wild type" in as many individuals and under as many circumstances as possible. The fact that all the alleles studied by us in *Harmonia axyridis* occur in natural populations suggests that they are at least tolerated by natural selection. Complete dominance and recessiveness have no biological meaning in such conditions. It does not follow, of course, that the color patterns and the alleles which influence them are adaptively neutral. It is possible that some of them are more favored in certain environments in which the species lives and others in other environments. In this respect the finding of TIMOFEEFF-RESSOVSKY (1940) is of interest—namely, that the dark variants of *Adalia bipunctata* are favored during the summer while the red variants are more favorable for survival during hibernations. Similar effects of natural selection on the incidence of various gene arrangements in different seasons have been established by DOBZHANSKY (1943) in *Drosophila pseudoobscura* and by DUBININ and TINIAKOV (1945) in *Drosophila funebris*. A careful study of the situation in *Harmonia axyridis* from this point of view is a matter for future work.

SUMMARY

The inheritance of elytral color patterns in the lady-bird beetle, *Harmonia axyridis*, has been studied. The material was collected in Meitan of Kweichow province, southwestern part of China. The inheritance of color patterns can be accounted for by assuming 12 alleles of an autosomal locus. The color patterns and the alleles responsible for them are *succinea* (*s*), *conspicua*-1 (S^{C1}), *conspicua*-2 (S^{C2}), *transversifascia*-3 (S^{T3}), *transversifascia*-1 (S^{T1}), *equicolor* (S^{E}), *spectabilis*-1 (S^{S1}), *spectabilis*-2 (S^{S2}), *intermedia* (S^{I}), *aulica*-2 (S^{A2}), *aulica*-1 (S^{A1}), and *tripunctata* (S^{R}).

Succinea homozygotes (*ss*) have yellow elytra with a varying number of black spots (0–19). The number and size of the spots present in homozygous *succinea* depend upon the temperature during the pupal development. All other variants of the elytral color pattern have a black background with yellow or orange spots.

The crosses between *succinea* homozygotes and each of the 11 other variants give rise to heterozygotes in which the *succinea* characters are recessive, except that the characteristic spots of the *succinea* parent may be found on the yellow areas.

Heterozygotes involving alleles other than *succinea* may be classified into two categories—namely, inclusive combinations and overlapping combinations. In the former, which result from combinations of two alleles where the black pigmented area characteristic of one in homozygous condition includes the black pigmented area produced by the other allele in homozygous condition, the pattern of the heterozygotes cannot be distinguished from that of the homozygotes having the more extensive black pigmented area. In the case of overlapping heterozygotes, which are combinations of two alleles where the black pigmented area of the pattern characteristic of one allele in homozygous condition overlaps the characteristic region of distribution of the other allele in homozygous condition, the pattern of the heterozygotes can invariably be distinguished from the patterns of either parental homozygous type.

The expression of the color pattern in the heterozygotes conforms to the rule of mosaic dominance, which states that any portion of the elytra which has black pigment in the homozygote for a given allele will have black pigment also in the heterozygotes in which that allele is present. The only exceptional individual which seems to break this rule shows the mosaic effects of three alleles—namely, S^E, S^R, and *s*. This individual might have been a trisomic.

The rule of mosaic dominance has been found equally applicable to the heterozygotes carrying three alleles which have been studied by HOSHINO (1940)—namely, *axyridis* (S^X), *transversifascia-2* (S^{T2}), and *forficula* (S^F).

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ABSTRACTS OF PAPERS PRESENTED AT THE DELAYED 1945 MEETINGS OF THE GENETICS SOCIETY OF AMERICA

St. Louis, Mo., March 28–30, 1946

LAURENCE H. SNYDER, *Secretary*
Department of Zoology and Entomology
The Ohio State University
Columbus 10, Ohio

Beers, Catherine V., and Estell A. Cheever, University of Southern California, Los Angeles, Calif.: *Hereditary ataxia.*——An ataxic gait is manifested in 18 males and two females, in six generations of a kinship including 162 individuals. Susceptibility has come from a common ancestor prior to generation 1. Fifteen out of 16 affected individuals each had an affected parent; therefore in this family an autosomal dominant gene with slightly reduced penetrance appears to be responsible for the susceptibility. Evidence from the critical marriages indicates that it may be a new partially sex-linked gene with a cross-over value of about 24 percent. Expressivity is variable. Eight of the affected males have received treatment for enlarged prostate glands. In this family there is no evidence of mental disorders, the ataxia is not due to syphilis and does not reduce the span of life. Other traits found in certain individuals in this kinship are: arthritis, muscle atrophy, cancer, high blood pressure, left-handedness, multiple births, and missing lateral incisors. None of these characters appears to be linked with ataxia. This ataxia may be eliminated if individuals in affected branches of the kinship refrain from having children. When preventive measures are found, treatment should be started as soon as premonitory symptoms are noted.

Blakeslee, A. F., Smith College Genetics Experiment Station, Northampton, Mass.: *Problems in plant embryo culture.*——A progress report of investigations being carried on by the group in the Smith College Genetics Experiment Station. Improvements in technique and applications in theoretical and applied plant breeding will be discussed.

Bogart, Ralph, University of Missouri, Columbia, Mo.: *Inheritance and physiology of dwarfism in sheep.*——Several lambs which are dwarf and die within one to two months after birth have been produced by one strain of South Down sheep. At birth the lamb is short legged, has a bulging forehead, and is thick through the shoulders. The palate and throat are abnormal, resulting in difficult breathing at all times and strangling at nursing. They gain

in weight at a slower rate than normal but become very fat and are consequently susceptible to high temperatures. Histological studies of the thyroid indicate a relationship of this gland with dwarfism. The colloid is lacking in 80 percent of the follicles and derangement of the cellular material is pronounced, with connective tissue invading the follicle cavity. Some parts of the gland apparently are functioning. Thyroxine therapy helped but did not completely alleviate the symptoms. The abnormality is a simple recessive lethal. There is apparently a close linkage of dwarfism with short under jaw. No evidence of sex linkage has been obtained.

BRAUCH, LIANE R., and W. L. RUSSELL, Roscoe B. Jackson Memorial Laboratory, Bar Harbor, Maine: *A study of the physiological genetics of coat color in the mouse by means of the dopa reaction.*——The dopa reaction in hair bulbs was studied in frozen sections of skin of 73 non-agouti and yellow genotypes. The results are similar to those found in the guinea pig. Qualitatively, the pigment produced resembles eumelanin, but the quantitative effect of each gene substitution on the reaction in both yellows and non-agoutis closely parallels the effect on yellow pigment. Thus, neither *bb* nor *pp* affect the reaction markedly and reduction by the lower members of the albino series matches yellows better than non-agoutis. Reactions in $A^{v}a$ and *aa* animals of corresponding genotypes are approximately equal. $W^{v}w$ dilutes the reaction only slightly. $W^{v}W^{v}$ prevents it completely. Maltese dilution, *dd*, intensifies the dopa reaction, a result which at first seemed opposite to the effect of *dd* on yellow pigment. See, however, the accompanying paper.

BRAUCH, LIANE R., and W. L. RUSSELL, Roscoe B. Jackson Memorial Laboratory, Bar Harbor, Maine: *Colorimetric measurement of the effects of the major coat color genes in the mouse on the quantity of yellow pigment in extracts*—— Alkaline pigment extracts of skin and hair of 150 yellow mice of 29 genotypes were compared in a photoelectric colorimeter. Substitution of *bb* for *BB* has no effect. Pink-eye (*pp*), however, probably reduces intensity in combination with $c^{ch}c^{ch}$ and Cc^{a}. In the albino series, *C* is dominant, except possibly in the compound Cc^{a}, and the last trace of pigment appears in $c^{ch}c^{a}$ ($c^{a}c^{a}$, $c^{e}c^{a}$, $c^{a}c^{a}$ being white in all backgrounds). Rather unexpectedly, Maltese dilution, *dd*, was found to *increase* the amount of pigment in all cases. This, however, agrees with the intensified dopa reaction reported in the accompanying paper and confirms the view that the dopa reaction parallels the yellow-producing process.

BURNHAM, CHARLES R., University of Minnesota, St. Paul, Minn.: *A gene for "long" chromosomes in barley.*——Several lines of Mars barley produced by X-ray treatment were found to have plants with 15 to 20 percent of pollen abortion. In one of these many of the chromosomes at metaphase I of microsporogenesis had the "long" appearance similar to that figured and described

by LESLEY and FROST (1927) in the Snow-flake variety of Matthiola. The number of such "long" chromosomes observed at metaphase I varied from none to six, the mode being at three to four, while the remainder appeared to be normal pairs. Two univalents were frequently observed at metaphase I as well as lagging chromsomes and micronuclei at telophase I. In two cases a lagging univalent had divided at this stage. The "long" appearance may be the result of failure of one of the two arms of a chromosome to be associated with its homologue. When such failure occurs in both arms, univalents occur. Whether the decrease in terminal chiasmata is compensated for by an increase in interstitial chiasmata as reported by ARMSTRONG and HUSKINS (1934) for the Matthiola case is not known. As in Matthiola, this character appears to be inherited as a recessive. The progeny of this plant all showed a similar degree of aborted pollen and, as far as examined, the same cytological behavior. At least two of the three normal sibs segregated for the same character. It is predicted that trisomic individuals will be found in the progeny.

CARSON, HAMPTON L., and H. D. STALKER, Washington University, St. Louis, Mo.: *Chromosome studies on Drosophila robusta.*——A preliminary survey has been made of the chromosome conditions in six recently collected stocks from widely distributed localities in the eastern United States. Further studies are in progress. In all six stocks, and in cross between three of them, six euchromatic arms and one dot extend from the chromocenter in the salivary gland cell nuclei; all except the dot are continuous through the chromocenter, suggesting that the metaphase configuration of the female includes three pairs of V's in addition to a pair of dots. The salivary X chromosome is medium in length. So far, metaphase configurations have been obtained for three stocks (Rochester, N. Y., Gray Summit, Mo., Philadelphia, Pa.); these agree with the salivary picture, showing three pairs of V's (one large, one medium, one small) and one pair of dots in both sexes. The Y chromosome is indistinguishable from the X. This chromosome group does not agree with previous descriptions for this species, which have indicated a salivary chromosome group of five arms and a dot and a metaphase complement of two pairs of V's, one pair of rods and a pair of dots. To date, eight different inversions have been found: four of these are in the X chromosome, two in each arm. Inversion heterozygosity within individual stocks is common. Tentatively, it appears that at least some of the inversions are wide-spread in natural populations; for example, one particular autosomal inversion has been found in all stocks so far studied.

CHASE, HERMAN B., University of Illinois, Urbana, Ill.: *Greying induced by X-rays in the mouse.*——Irregularities in degree and pattern described by previous workers have been here controlled by obtaining groups of hairs at known stages of development and by using hard X-rays in a phantom to give homogeneous dosages to the whole animal. The inbred C57 Black strain has been used. The threshold dosage is 250 r and about 50 percent loss of pigment occurs

with 500 r. A higher dose of 1000 r using soft rays on a prepared area gives 100 percent loss of color. Plucking of hair on areas of 1.5 square centimeters is essentially the method of obtaining new hair generations. Time of X-raying relative to times of plucking makes it possible to have on same animal several areas differing as to hair age. Maximum effect on the hair itself is when hair is growing rapidly; maximum effect on loss of pigment is instead at time preceding hair growth. Effect is on the reservoir of melanophores. This standardized method on C57 Blacks gives permanent readings within three weeks and has permitted biological assays of radiations from different sources (radium, betatron) and has been used in comparative studies on different genotypes and phenotypes.

CHASE, HERMAN B., University of Illinois, Urbana, Ill.: *A sex-linked recessive in the mouse.*——Polydactyly occurred in our colony. Variations in expression agree generally with those found by FORTUYN (Genetica 1939). In matings of polydactyl by polydactyl, the frequency of expression is about 25 percent. Data from reciprocal outcrosses and subsequent matings indicate a single sex-linked recessive as the simplest explanation. This is the first case of sex-linked inheritance in the mouse.

CHILD, GEORGE P., University of Georgia Medical School, Augusta, Georgia: *The effect of delaying time of development on mutation rate.*——The duration of the larval period of *D. melanogaster* was extended to 30–40 days at 25 degrees. This was accomplished by allowing the larvae to develop on molasses-agar with one percent nipagin and no yeast. The mortality was very high in both larval and pupal periods. The few flies which did finally emerge were very small, weak, and relatively infertile. These flies were tested for lethals in the second chromosome by the usual methods. Only 56 such tests were made and six lethals were found, three from one parent and one each from three other parents. This frequency of mutation far exceeded the rate found in the more extended control series.

DICKERSON, G. E., and J. W. GOWEN, Regional Swine Breeding Laboratory and Iowa State College, Ames, Iowa: *Food utilization in genetic obesity of mice.*——The marked obesity induced in mice by the "yellow" gene provided opportunity to test the validity of an observation in swine that heritable increases in rate of fat deposition reduced food requirement per unit gain. Yellow (*Y*) and black agouti (*N*) segregates were obtained by crossing *Y*♂'s of one highly inbred stock with albino ♀'s of another. Amount and composition of gain and food consumption of *Y* and *N* littermates were compared in four replications within each sex and period, periods being from 25 days to ages of 30, 50, 90, 200, and 300 days. Mice were self-fed to permit full expression of genic effects on appetite. From 25 to about 40 days, *Y* and *N* were alike in amount and composition of gain and in feed consumption. From 40 to 200

days, *Y* exceeded *N* in gain by 2.1× in ♂'s and 2.5× in ♀'s, but in feed consumption *Y* exceeded *N* by only 1.24× in ♂'s and 1.15× in ♀'s. Weights at 200 days were: *N* ♀, 28 gm; *Y* ♀, 47 gm; *N* ♂, 33 gm; *Y* ♂, 48 gm. The excess gain of *Y* in both sexes was entirely fat tissue; however, ♂'s exceeded ♀'s by nearly one-half in protein gain. Feed per gram gain, and especially, per calorie of gain, was lower for *Y* than *N* at any age beyond about 40 days. Feed per unit gain, however calculated, was lower for ♂'s than for ♀'s, particularly for *N*'s and at ages up to about 90 days. During weight constant periods, feed per unit of live weight was less for *Y* than for *N* by one-fourth in ♀'s and by one-seventh in ♂'s, but was similar for *Y* ♀'s and ♂'s. Activity was much less for *Y* than *N*, particularly for ♀'s. The yellow gene produces obesity by increasing appetite and reducing energy requirements.

EYSTER, H. C., University of South Dakota, Vermillion, South Dakota: *Inheritance in the petunia.*——The inheritance of several traits has been investigated and determined. Hybrids have definitely proven their superiority over inbred lines.

EYSTER, H. C., University of South Dakota, Vermillion, South Dakota: *Theoretical aspects of hybrid corn genetics and hybrid vigor.*——Some interesting theoretical mathematical aspects have been worked out. These could be the basis for further research in an effort to learn more about the nature of hybrid vigor.

FOGEL, SEYMOUR, University of Missouri, Columbia, Mo.: *Gene action and histological specificity of pigmentation patterns of certain R alleles.*——The gene *R* is known to affect the occurrence, extent, and intensity of anthocyanin pigmentation in the gross organs of the plant (Records of the Genetics Society of America **11**:90–91, **12**:36–37). The highly differentiated tissues and the wide diversity of cell types within a single tissue provide in effect an unlimited series of reagents for the study of specificity of genic action. Several alleles of divergent action, *R*-pueblo, *R*-cornell, *r*-cornell, and *r*-ch, in all possible test cross combinations of the type $R^r/r^r \times r^g$, were selected for study of the pattern of pigment development. With respect to pigmentation of specific cellular types, the alleles are seemingly linearly seriable in the decreasing order of potency: *r*-ch, *R*-cornell or *r*-cornell, *R*-pueblo. This is substantially the case for the cell types of the seedling leaf margin, mesocotyl, coleoptile, and sheath. Within the coleoptile, however, *R*-pueblo plants manifest pigmented stomatal guard cells. This cell type is unpigmented in *r*-cornell or *r*-ch sibs. In their effects on pigmentation of the leaf collar, the order of potency is: *R*-pueblo, *r*-ch, *R*-cornell and *r*-cornell. *r*-ch, exhibiting a moderate deposition of pigment restricted to the abaxial epidermis, stands in contrast to *R*-pueblo which shows not only an increased frequency of more intensely colored cells in both epidermal layers, but also exhibits pigmentation of internal cellular types—for

example, mesophyll and bundle sheath cells.—By introducing specific modifiers, by subjecting plants to temperature shocks, or by maintaining excised tissue sections on buffered glucose solutions, it is possible to induce anthocyanin synthesis in tissues where it is not normally found. This provided further possibilities for specificity studies.—With low night temperatures, anthocyanin appeared in the leaf of appropriate genotypes. Superficially, *R*-cornell and *r*-cornell showed but a faint trace of pigment in the abaxial epidermis, *r*-ch an intense coloration of both surfaces, and *R*-pueblo a moderate deposition in the adaxial epidermis. While *r*-ch and *R*-cornell exhibit pigmentation in several cell types common to both alleles, some 17 cell types are pigmented in the adaxial surface of *r*-ch and these are not pigmented in *R*-cornell. *R*-pueblo and *r*-ch share in common a different set of pigmented cells in the adaxial surface. Moreover the three cell types resistant to pigmentation in *r*-ch tissue, the horn cell, guard cell, and socket cell, are intensely colored in *R*-pueblo tissue. *R*-pueblo and *r*-cornell have no pigmented cell type in common.

FOGEL, SEYMOUR, University of Missouri, Columbia, Mo.: *Gene action and the course of anthocyanin synthesis in certain R alleles.*——In the presence of appropriate complementary factors, the amount of anthocyanin synthesized in excised tissue sections maintained on buffered glucose solutions is a function of *R* action. The anthocyanin is completely extractable from the tissue and may be quantitatively estimated as the chloride by colorimetric methods. Crystalline chrysanthemin chloride, prepared at this laboratory, was used as a primary standard. In cultured mature leaf sections a linear time course of synthesis is established after a 24 hour induction phase. The time course of synthesis has been determined for the alleles *R*-pueblo, *R*-cornell, *r*-cornell, and *r*-ch, in all possible test cross combinations of the type $R^r/r^r \times r^a$. Each allele may thus be characterized by a rate constant derived from the slope of the curve. With regard to rate and total amount of anthocyanin formed the alleles may be seriated in the decreasing order: *r*-ch, *R*-pueblo, *R*-cornell, and *r*-cornell.

GILES, NORMAN H., JR., P. R. BURKHOLDER, ILDA MCVEIGH, and KATHERINE S. WILSON, Yale University, New Haven, Conn.: *Comparative studies on the B-vitamin content of trisomic and disomic maize.*——Assays of leaf tissue for three B-vitamins (niacin, riboflavin, and thiamin) have been made of trisomic and comparable disomic plants of maize, including nine of the ten possible extra-chromosomal types. Essentially comparable results were obtained in assaying dried material collected 50 days after planting and fresh material collected 75 days after planting. In all but a few instances, the average vitamin content was higher in the trisomics than in the comparable diploids. The increases were relatively slight for certain extra-chromosomal types, but pronounced for others. The most striking increases were found in the following instances: (1) niacin, chromosomes 3, 8, and 9; (2) riboflavin, chromosomes 3,

5, 8, and 9; (3) thiamin, chromosomes 5 and 8. These results indicate that the addition of an extra dose of almost any chromosome has some effect in increasing the content in the leaf cells of the vitamins studied, which suggests that several genes may be involved in the synthesis of each vitamin and that these genes are widely distributed in the chromosome complement. Certain chromosomes, however, are clearly more important than others in causing an increased content of any particular vitamin. Furthermore, the fact that trisome 8 shows a considerable increase for all three vitamins suggests that this chromosome may carry genes controlling certain basic reactions common to the synthesis of all three substances.

GOODSPEED, T. H., University of California, Berkeley, Calif.: *Karyotypes, meiotic behavior, and systematics in Nicotiana.*——External morphology and distribution divide the genus into three subgenera and 11 sections. Distinctions in karyotypes between many sections and similarities within them are conspicuous, while amphidiploids reveal the chromosome contributions of their putative parents. In 135 F1 interspecific hybrids pairing at MI is lacking, low or high variable, "Drosera scheme" or complete. Whereas 90 percent of F1 intrasectional hybrids show approximately complete pairing, practically all intersectional and intersubgeneric ones show negligible pairing. All F1 hybrids between amphidiploids and their ancestors show "Drosera scheme" behavior, while 85 percent of F1 amphidiploid×other species exhibit almost complete lack of pairing. There is, therefore, a high degree of correspondence between a taxonomic arrangement based upon morphology and distribution and one which could be derived from cytogenetic evidence.

GOODSPEED, T. H., University of California, Berkeley, California: *Meiotic prophase studies in Nicotiana.*——Comparative studies of meiotic prophase in species of Nicotiana, in an asynaptic variety and in F1 interspecific hybrids indicate that extent of MI pairing is accurately reflected in extent and character of pachytene association. Irregularities of the prophase sequence in unpaired chromosomes resulting from alterations in normal timing of contraction, spiralization, and chromatid separation are evidenced in thick and thin segments at early pachytene, precocious initiation of spiralization in some segments at later pachytene and diplotene separation of sister chromatids. Whether these alterations are the product of failure of pairing or of genic unbalance remains to be determined. Investigation of prophases in hybrids with negligible pairing, in haploids and asynaptic plants can yield significant evidence concerning fundamental problems of chromosome behavior in meiosis.

GOWEN, JOHN W., Iowa State College, Ames, Iowa: *Significance of additive, dominant, complementary gene action to hybrid vigor in Drosophila.*——Earlier work on hybrid vigor in Drosophila has shown that chromosomal rather than cytoplasmic behavior is responsible for the observed results. The chromosomal effects may be analyzed by the use of reciprocal crosses between several

inbred lines and by successive backcrossing of hybrids to their parental inbreds for several generations with selection. The results of these studies show that additive gene action will account for most of our hybrids' increase in yield performance. Dominance, complementary gene action, etc., account for but a small proportion of the variance in the different groups. The genes for these results appear in all chromosomes and are distributed according to the quantity of chromatin material.

HOVANITZ, W., University of Michigan, Ann Arbor, Mich.: *Comparative dispersion of female color types of Colias.*——The orange and white females in a subpopulation of *Colias eruytheme* were marked during a three day period in October 1941 in order to determine if a difference in dispersion was present in these genetic types. This habit would be manifested in a divergent percentage of recovered as compared with first-captured insects, since the type that dispersed more rapidly would be represented by fewer individuals in the recovered lot. The frequency of the two phenotypes in the population was the same in the unmarked as compared with the marked individuals—28.7 percent white in the former (N = 272), 26.7 percent in the latter (N = 135), and 29.7 percent for the population as a whole for this period (N = 1,036).

HUBBS, CARL L., and LAURA C. HUBBS, Scripps Institute of Oceanography, La Jolla, Calif.: *Breeding experiments with the invariably female, strictly matroclinous fish, Mollienisia formosa.*——By 14 years of experimental breeding the preliminary conclusions regarding the genetic behavior of this poeciliid have been confirmed. About 8,000 young, representing 20 generations, have been produced. With the possible exception of one fish with rudimentary gonad, every specimen has been shown by dissection to be a female. No trace of paternal inheritance was detected, although the presumably gynogenetic fish were successfully mated with 50 types (species, subspecies, races and hybrids) of Mollienisia and with males of five species of other genera of the same tribe. Offspring were even obtained by crossing with Gambusia (of a distinct subfamily), principally by aid of pituitary injections and artificial insemination. When masculinized by hormone treatment, these offspring showed only Mollienisia characters in the gonopodium (which exhibits the most valuable systematic characters). All attempts to produce young without the intervention of male poeciliids failed, and the fecundity was roughly proportional to degree of relationship. Therefore development seems to be induced by sperm, though all the evidence favors the view that the offspring carries no paternal chromatin. In many other crosses in the tribe Poeciliini almost no indications have been found of either alternative inheritance or dominance. Critical cytoplasmic observations have not been secured, for chromosome counts in fishes have been made only for spermatogenesis, and all efforts to transform the ovaries into testes have failed. The most plausible explanation for the genetic behavior of *Mollienisia formosa* is that this unisexual species is a permanently fixed diploid.

HUESTIS, R. R., University of Oregon, Eugene, Oregon: *Linkage of flexed tail and albinism in Peromyscus.*——Each of four tests based upon a total of 305 mice shows linkage of flexed-tail and albinism. In female mice there was 30 percent crossing over in repulsion phase, 40 percent in coupling phase. In males there was 29 percent crossing over in repulsion phase, 33 percent in coupling phase. Standard errors range from 4 to 7 on these crossing over percentages.

HULL, FRED H., Florida Agricultural Experiment Station, Gainesville, Florida: *Regression analyses of corn yield data.*——Analyses of published data on yields of inbred and hybrid corn (one unpublished set from G. H. STRINGFIELD, Ohio Exp. Station and U.S.D.A.)show regressions of F1 on inbred parent in groups of one constant parent to be generally positive. These partial regressions are large when the constant parent is a low yielding and approach zero when the constant parent is a high yielding line. Theoretically, with additive gene action and complete dominance but no "overdominance" ($aA > AA$), level regression is not obtained until the constant parent is AA at all loci and equal to any hybrid. M. T. JENKINS' data (J. Agric. Res. **39**: 677–721: tables 17 and 19) seem to represent nearly flat regressions surfaces. Partial regression coefficients for each row and each column of F1 on parallel row or column of P are all small, averaging approximately zero. The best interpretation seems to be gene frequencies near equilibrium values at many loci where the heterozygote has superior selective value—see FISHER, R. A., "Genetical Theory of Natural Selection." Other data examined do not present level regression surfaces. No other set seems to represent corn so nicely adapted by selection in open-pollinated varieties as the two sets in JENKINS' tables 17 and 19. Some slight degree of multiplicative gene action is indicated by trend of partial regression with respect to constant parent in some sets of data examined. Failure of ear-row selection and Fn of multiple crosses of *selected* lines are inconsistent with classical dominance theory of heterosis but not with a theory of overdominance.

HUNT, H. R., and C. A. HOPPERT, Michigan State College, East Lansing, Mich.: *Inheritance of susceptibility to dental caries in rats (Mus norwegicus).*——Hereditary differences in susceptibility to dental caries have been demonstrated in rats fed the caries-producing diet of HOPPERT, WEBBER, and CANNIFF. The animals are placed on this diet when 35 days old. The lower molar teeth are examined for caries every 14 days. A susceptible and a resistant line have been produced by phenotypic selection, selection of breeding pairs by the progeny test, and brother×sister inbreeding. A total of 4,557 rats have been studied to date. The first (unselected) generation developed caries, on the average, at 70 days. The average for the 14th generation of susceptibles is 18 days; for the (incomplete) ninth generation of resistants it is 339 days. The susceptible line now has low variability and is probably nearly homozygous. The

resistant strain shows very high variability. There is some evidence that variability in the resistants is affected by mechanical breakage of the upper molars. When low variability is attained in both lines, an attempt will be made to estimate the number of pairs of genes involved.

IRWIN, M. R., and R. W. CUMLEY, University of Wisconsin, Madison, Wis.: *A second genetic analysis of species differences in Columbidae.*——It has already been reported that Pearlneck (*Streptopelia chinensis*) differs from Ring dove (*St. risoria*) in ten or more antigens of the red blood cells, these having been obtained as individual antigens. By the same techniques, cellular characters peculiar to Senegal (*St. senegalensis*), in contrast to Ring dove, have been obtained in unit-form in backcross progeny to Ring dove of the species hybrids and selected backcross hybrids. There are at least nine, probably ten, of these substances specific to Senegal. They are called s-1, s-2, s-3, s-6, . . . s-12, to distinguish them from the antigens of these two species—Pearlneck and Senegal.

IVES, PHILIP T., and MARY B. ANDREWS, Amherst College, Amherst, Mass.: *Analysis of the sex-linked mutation rate in the Florida "high" stock.*——The high mutation rate in this stock is caused by a recessive second chromosome gene. The stock is maintained in several *Cy*/lethal lines, heterozygotes for any two such lethals being homozygous for "high." X-lethal mutation rates were determined in four series of such heterozygotes, involving lines number 1, 5, 14, and 29. Individual males composed of lines 1/5, 5/29, and 14/29, mated continuously, showed a drop in the proportion of X-lethals found in sperm deposited during four consecutive weeks, the percentile rates being, respectively, 1.31 (1527 tests), 0.59 (1351 tests), 0.12 (1614 tests), and 0.26 (1171 tests), not including duplicate lethals. In 5/14 males the rate remained high: 0.70 (713 tests), 1.31 (764 tests), 1.18 (932 tests) and 1.30 (923 tests), suggesting that lines 5 and 14 represent a different allele than do 1 and 20. In the crosses involving 1 and 29 the effects of "high" may be earlier than the first week of adult life, since 5/29 males slowed by ten to 20 days in development at 25°C by the use of yeast-deficient food gave but two lethals in 2013 tests. It is suggested that X chromosome lethals, being homozygous, shorten the reproductive life of spermatogonia, and that the observed X-lethal mutation rate in males depends upon the balance between this factor and the rate of new mutations.

JAAP, R. GEORGE, Oklahoma A. and M. College, Stillwater, Okla.: *Sexual dimorphism in natal color patterns of the domestic fowl.*——Using two common breeds of the domestic fowl, the Rhode Island Red and the White Plymouth Rock, sexual dimorphism in the natal color pattern has been greatly intensified. The relation between these and other dimorphic color patterns of the chick will be illustrated by means of prepared skins. From preliminary ob-

servations on six strains of Rhode Island Reds it was observed that the most common color difference between male and female chicks was the presence or absence of a creamy white splash on the dorsal wing surface. Chicks were selected in which the males had a distinct white area and the females a uniform "red" color on the dorsal surface of the wing. On one generation of selection, accuracy in identifying sexes by this method was increased from 88 to 95 percent. The natal color of the White Plymouth Rock may exhibit a certain amount of melanism. By selection this melanism has been increased to an intensity approaching that of the Barred variety. Utilizing the dimorphic action of the barring gene, a color discrepancy between the sexes has been produced in this variety which normally lacks color dimorphism. The two types of color dimorphism to be presented are cumulative when combined in a variety with colored plumage. This is distinctly evident in the "gold-barred" and Creole patterns. When barring is combined with the silver allele of the gold or "red" plumage, sexual dimorphism is also very great. Whether sexual dimorphism may be developed in the natal color of the non-barred silver phenotype remains for further investigation.

KRUGELIS, EDITH J., Vassar College, Poughkeepsie, New York: *Intracellular alkaline phosphatase activity.*——The histo-chemical test for alkaline phosphatase in mouse tissues produced different intracellular localization of activity when different organic phosphate esters were used as substrates. With sodium glycerophosphate and the mononucleotides (adenylic acid, guanylic acid, cytidylic acid) as substrates, the location of the activity of alkaline phosphatase occurred in the cytoplasm and the nucleus (chromosomes and nucleolus). With polymarized desoxyribose nucleic acid as substrate, no reaction occurred, but with depolymerized desoxyribose nucleic acid as substrate, the location of the activity was almost exclusively in the nucleus. With ribose nucleic acid as substrate, the location of the activity occurred mainly in the cytoplasm. These three different types of reactions suggest the presence of three intracellular enzyme complexes, whose activity depend on the substrate applied. The coincidence of the location of the activity of alkaline phosphatase on depolymerized desoxyribose nucleic acid with the general location of this substrate in the cell, and the location of the activity of alkaline phosphatase on ribose nucleic acid with the general location of this substrate in the cell, strongly suggests a functional relationship of alkaline phosphatase and the nucleic acids in the cell.

LANGHAM, D. G., Instituto Experimental de Agricultura, Caracas, Venezuela: *Variations in the sesame flower.*——A recent study of the flowers of forty-four varieties of sesame (*Sesamum indicum L.*) from many different countries and hybrids among them, has revealed 33 morphological differences and 30 color differences. These numbers include only the basic types and not the numerous combinations of them. A series of multiple alleles and many

cases of linkage (the first to be described in sesame) have been uncovered in a genetical study of some of these characters. Of particular interest is the specific distribution of the colors in the flower, indicating that a histological study of this organ would be fruitful. The striking characteristic difference in the flowers of varieties obtained from various geographic regions suggest this as a good starting point for a study of the origin of sesame.

LANGHAM, D. C., Instituto Experimental de Agricultura, Caracas, Venezuela: *Open sesame.*——The magic words "Open Sesame," used in one of the well known stories of the "Arabian Nights," are based on the extreme dehiscence of the seed pod of sesame (*Sesamum indicum L.*), one of the oldest cultivated oil crops in the world. This important source of edible vegetable oil has not become an established commercial crop in the Western Hemisphere because of difficulties in mechanical harvesting. The indeterminate growth habit, coupled with the dehiscence of the pod, necessitate considerable hand labor at a specific time to gather the crop. An indehiscent mutant found recently in the breeding plots of the Agricultural Experiment Station in Venezuela may be the key to the solution of the problem. This new type behaves as a simple recessive in crosses with dehiscent sesame.

LAUGHNAN, JOHN R., University of Missouri, Columbia, Mo.: *Chemical studies concerned with the action of the gene A_1 in maize.*——As determined visually, the A_1 allelomorphs are concerned with the production of varying amounts of purple and brown pigments. *A B Pl* plants are pigmented deep purple in most external tissues, while *aa B Pl* individuals are brown and without anthocyanin in these regions. Chemical studies carried out with extracts of husks from brown *aa B Pl* plants have established the presence of at least five chemically distinct pigments. The latter are phenolic in nature, retain considerable brown color in acid solutions, and all are absent in purple *A B Pl* plants. Brownhusk extracts also contain two yellow pigments which are colorless in acid solutions and have the properties of flavonols. Chemical and spectrographic absorption studies have shown one of these (Flavonol II) to be identical with isoquercitrin; the other (Flavonol I) is structurally unidentified. Both yellow pigments are also present in *A B Pl* husk extracts but to considerably reduced extent. Extracts of purple husks of *A B Pl* plants contain at least two purple pigments in addition to the anthocyanin, chrysanthemin. There are indications of others from preliminary studies. The purple pigments are distinct from the brown and yellow pigments and are absent in husk extracts of *aa B Pl* plants. The *A* gene may thus be viewed as affecting the synthesis of a relatively large number of related chemical substances. It appears from the present study that there is little basis for the generally accepted view that the action of this gene is concerned with a simple oxidation-reduction step which makes the differences between isoquercitrin and chrysanthemin.

LESLEY, MARGARET, and J. W. LESLEY, University of California Citrus Experiment Station, Riverside, Calif.: *A genetic study of flesh color of fruit in*

hybrids of Lycopersicon esculentum Mill. and L. hirsutum Humb. and Bonpl.—— Red fleshed *RR* or *Rr* plants of *L. esculentum* were crossed with *L. hirsutum* having yellowish green fruits. F1 was back crossed to *esculentum* (*RR*). The various flesh colors were compared with the colors in MAEZ and PAUL's Dictionary of Color. Very little red was present in the F1 fruit. In BC 1, BC 2, and derived families at least six types of flesh color occurred not found in either of the parental species. They varied in intensity of red and yellow colors. In some types most of the pigment was β-carotene, and only a little was lycopene. No *rr* (yellow fleshed) or *tt* tangerine types of *esculentum* occurred. As a rule, types deficient in red color are dominant. A type termed orange, with pink flesh (M. & P. 3H11), is probably a simple dominant to the red of *esculentum*. The species differ in at least three genes whose dominant alleles inhibit the formation of pigments, especially lycopene. *L. hirsutum* and the F1 plants rarely set fruit, although chromosome behavior in the p.m.c. was fairly regular. Many plants in BC 1 and certain later generations were unfruitful.

LINDEGREN, CARL C., Washington University, St. Louis, Mo.: *Cytoplasmic effect and chromosome maps in Saccharomyces cerevisiae.*——Yeast hybrids have been produced heterozygous for genes controlling mating type, galactose and melibiose fermentation, auto-agglutination of the cells in broth, and some additional colonial characters. Some of these genes were found to be linked. Cytoplasmic transfer of cytogenes complicates genetical analysis of hybrids heterozygous for genes controlling the fermentation of galactose, melibiose and the synthesis of vitamins. In some crosses cytoplasmic transfer of cytogenes obscured the mendelian inheritance, but by making other matings, it has been possible to get clear cut mendelian segregations. Matings of the latter type have revealed that fermentation of galactose is controlled by a single locus.

LUCE, WILBUR M., University of Illinois, Urbana, Ill.: *Effects of temperature on adorso-central (adc), a new bristle reducing mutant of Drosophila melanogaster.*——Flies carrying adorso-central (*adc*), a sex-linked recessive in *Drosophila melanogaster*, which is characterized by the loss of dorso-central bristles, were raised at constant temperatures of 17, 22, 24, and 28 degrees C. At 17 degrees, less than one percent of the flies lacked dorso-centrals, while at 28 degrees 72 percent of the females and 88 percent of the males lacked one or more of these bristles. At 22 degrees, the percentages which lacked bristles were six for the females and 22 for the males. At 24 degrees, 14 percent of the females and 28 percent of the males lacked one or more bristles. These results show that the percentage of flies showing the characteristic loss of bristles is higher in males than in females at all temperatures except 17 degrees. A preliminary experiment to determine the critical period for the temperature effect at 28 degrees indicated that this period begins near the end of the fourth day of larval life and extends well into the pupal period.

MACBRIDE, DOROTHY H., University of Maine, Orono, Maine: *Failure of sperm of Habrobracon diploid males to penetrate the eggs.*——Fifteen diploid males, tested in 42 crosses, proved sterile, 692 haploid impaternate males but no daughters developing. Abdomens of females were fixed, stained (Delafield's hematoxylin and eosin), and sectioned. Of seven females mated to haploid males, four showed spermathecae full of sperm; one had few sperm; two resembled unmated females with sperm neither in spermatheca nor in the adjoining duct. Of 19 mated to diploid males, numbers in the three groups were 5, 7 and 7, respectively. Living sperm from vasa deferentia of diploid males, placed in Ringer's solution, resembled those from haploid, exhibiting constant and rapid circular motion by waving of the long tail piece. Eggs of females mated to diploid males and kept at 26°C were fixed (Kahle) one-half hour after oviposition and stained (Feulgen). Of 29 in good condition for study showing polar bodies and egg nucleus in second maturation or first cleavage, only one showed a possible, but questionable, sperm nucleus. The sterility or very low and infrequent fertility of diploid males long noted in the literature, therefore, appears due to failure of the diploid sperm to penetrate the egg. Number of flagellar segments of 159 antennae of haploid males ranged from 18 to 21 with frequencies—1, 12, 135, 11, while for diploid males range was 15 to 20 with frequencies—1, 1, 13, 51, 85, 8. In this stock, No. 11, as in others previously tested, diploid males average fewer segments and their spread is greater.

MCGIBBON, W. H., University of Wisconsin, Madison, Wis.: *A sex-linked antigen of mallard ducks.*——The red blood cells of reciprocal duck hybrids produced by matings between representatives of the Mallard (*Anas platyrhynchos*) and Muscovy (*Cairina Moschata*) species have been assayed (using the agglutinin absorption technique) with respect to those antigens specific to Mallard and not shared with Muscovy. Several individual differences in antigens were noted among the hybrids. One of these characters of Mallard was possessed by all F1 ♂♂ produced by Muscovy ♂♂ × Mallard ♀♀, while none of the F1 carried it. The cells of all F1's, irrespective of sex, resulting from mating Mallard ♂♂ × Muscovy ♀♀ contained this substance. From these results, it would appear that the gene or genes conditioning this character are sex-linked in Mallard. The inability to fractionate the antibody detecting this substance suggests its unitary nature but does not necessarily establish it.

MIRSKY, A. E., A. W. POLLISTER, and HANS RIS, Rockefeller Institute for Medical Research and Columbia University, New York: *The chemical composition of chromosomes.*——Mass preparations of isolated nuclei and isolated chromosomes have served as the material for most of these investigations. The substances which make up the bulk of a chromosome fall into three groups: desoxyribose nucleic acid, histone, and non-histone protein. The properties and compositions of histone and non-histone protein differ so much from each other that it is possible to separate them and to distinguish one from

the other under various conditions. Since we find that histones contain no more than traces of tryptophane and since the non-histone protein fraction contains a fair quantity of tryptophane, we refer to the latter as TrPr (the tryptophane-containing protein fraction of chromosomes). When isolated, both histone and TrPr are able to combine with nucleic acid in a neutral medium; and in chromosomes both proteins are combined with nucleic acid. From nuclei and chromosomes nearly all of the histone and the nucleic acid attached to it can be removed either enzymatically or by use of concentrated neutral salt solutions. The residual material, which consists essentially of TrPr and the nucleic acid attached to it, exhibits the microscopic structure characteristic of the nucleus or chromosome from which it was derived; the reactions to stains of the residue are, however, quite different from that of the original material. The relative quantities of nucleic acid, histone and TrPr vary considerably in chromosomes derived from different types of cells. Methods have been worked out for the quantitative identification of these constituents in microscopic preparation.

MULLER, H. J., Indiana University, Bloomington, Ind.: *Physiological effects on "spontaneous" mutation rate in Drosophila.*——The over-all mutation frequency at constant temperature is often considered proportional to time (age), as some (not all) previous Drosophila data were thought to indicate. The present experiments, involving tests of some 200,000 X chromosomes for "spontaneous" lethals, demonstrate, on the contrary, that the time rate of mutation varies greatly among different types of normal cells, representing different stages in the germinal cycle. Specially constructed stocks were employed that increased the efficiency of the testing and allowed chromosomes from both male and female parents to be tested simultaneously. Over-all mutation frequency is two or three times higher in the first crop of spermatozoa, accumulated during a male's pre-imaginal life, than in those of a week later, derived from a non-virgin male. This proves the existence of special physiological conditions increasing mutation rate at some stage in the development or mature life of the former while not (or less) affecting the latter cells, simultaneously present. With storage of spermatozoa in the female (and probably in the male) their mutation frequency rises considerably, though insufficiently to explain the high frequency from young males on the basis of storage in pupae. Chromosomes from females show practically constant frequency, regardless of parental age or metabolic activity. Hence most of their mutations must occur at some definite stage or stages, very few during "ageing" of oögonia. One such sensitive stage proved to be early cleavage. The results may help explain physiologically the relatively low time rate of mutation in long-lived species. Structural changes showed very low frequency.

MYERS, W. M., U. S. Regional Pasture Research Laboratory, State College, Penn.: *Effects of cytoplasm and gene dosage on expression of male sterility in Dactylis glomerata.*——A type of male sterility found in *Dactylis glomerata* is

dependent for its expression upon the interaction of a cytoplasmic condition with a dominant gene. In crosses of the sterile (male sterile) parent with various fertile plants, the F1 consisted of all sterile or of sterile and fertile plants in different proportions, depending upon the genotype of the fertile parent. For genetic analysis, 6,483 plants were classified from backcross and test cross progenies of F1 plants of three families. The results were consistent with the hypothesis that plants from seed produced on sterile parents were sterile if quadruplex, triplex, or duplex for the sterility gene, fertile if nulliplex, and either sterile or fertile if simplex. Among F1 plants used in test and backcrosses, seven proved to be simplex. Of these, three were fertile and four were sterile. Ratios observed in most test and backcross progenies agreed well with those calculated on the assumption that simplex plants were sterile and fertile in equal frequency. The few exceptional ratios could be explained by the assumption of modifying factors that changed the proportion of sterile and fertile plants in the simplex class. The fertile parents used in two crosses proved to be triplex, indicating that the gene does not condition male sterility in absence of the appropriate cytoplasm. That the effect was cytoplasmic, not maternal, was indicated by recovery of male sterile plants in expected frequency from intercrossing two fertile F1 plants known from other breeding tests to be simplex.

OAKBERG, EUGENE F., Iowa State College, Ames, Iowa: *Constitution of liver and spleen as a physical basis for genetic resistance to mouse typhoid.*——Six strains of mice representative of low, intermediate, and high genetic resistance to mouse typhoid were inoculated intraperitoneally with a dose of 200,000 live *Salmonella typhimurium* organisms from a culture with moderate virulence. Earlier work had shown that leucocyte numbers are significant elements in differentiating resistant from susceptible mice. In this work the bases for these resistance differences were extended to include significant changes in liver and spleen. Necropsy data indicated that susceptible animals developed moderate liver lesions and moderate-to-severe necrosis of the spleen; that individuals of intermediate resistance showed moderate liver lesions and almost no spleen lesions; and that resistant mice exhibited severe liver necrosis and very few spleen lesions. Thus lesion patterns of liver and spleen are characteristic of genetic constitution of the host. Histopathological studies revealed normal liver glycogen storage in resistant mice, while animals with intermediate and low resistance often showed very little liver glycogen after the onset of morbidity. Fatty infiltration of the liver was common in all strains. Counts of bacteria within reticulo-endothelial cells of liver and spleen indicated that the ability of macrophages to digest phagocytosed bacteria, and the normal glycogen content of hepatic cells of resistant mice, may be manifestations of cellular resistance to *S. typhimurium* toxins. Genetically resistant mice possess morphological and metabolic properties of liver and spleen that distinguish them from susceptible individuals.

OWEN, RAY D., University of Wisconsin, Madison, Wis.: *Erythrocyte mosaics among bovine twins and quadruplets.*——The high incidence of identity of blood types between bovine twins, unexpected in view of the low frequency of monozygotic twinning and the large number (40) of inherited cellular antigens known to be segregating in cattle, has been explained as resulting from cellular interchange between fraternal twin embryos. It appears that embryonal cells originating in a co-twin may become established in the hemapoietic tissues of an animal and continue to provide a source of blood cells distinct from the animal's own throughout his life. The population of blood cells of such an animal therefore represents a genetic mosaic of two different cell-types.—Direct evidence of this mosaicism has been obtained by fractionating cell suspensions from bovine twins and quadruplets, using a technique of differential lysis. No twin has shown more than two cell-types, but a mixture of three cell-types has been demonstrated in each member of a set of quadruplets.—Opportunity for an intermixture of bloods is offered by the well-known vascular anastomoses in the placentae of bovine twins. The extent to which tissues other than blood may depend upon primordial cells circulating in embryos, and may therefore show a similar mosaicism associated with multiple births in cattle, remains an open question.

RILEY, HERBERT P., University of Kentucky, Lexington, Ky.: *Chromosome aberrations in a natural population of Tradescantia paludosa.*——Plants of *Tradescantia paludosa* from Micheaud, Louisiana, were studied for chiasma frequency, extent of failure of pairing and frequency of univalents, and spontaneous chromosome fragmentation at the first meiotic metaphase. The frequency of inversion bridges with or without accompanying acentric fragments and the relative sizes of such fragments at first anaphase were determined. Exceptional behavior at the microspore division and pollen fertility were noted. A comparison of this population with one of approximately equal size about 20 miles away and on the opposite side of the Mississippi River shows little fundamental difference between the two populations. In both, the percentage of inversion bridges and other abnormalities was high, indicating that there is structural hybridity in a very large number of individuals of this species.

RUSSELL, ELIZABETH S., Roscoe B. Jackson Memorial Laboratory, Bar Harbor, Maine: *A quantitative study of genic effects on coat color in the house mouse.*——From tip to base a fully grown hair contains a serial account, through the active life of the follicle, of the type, extent, and variation of the pigmentation processes occurring there. Detailed study of the pigment granules in cross-sections and at various levels in whole mounts of 36 genotypes indicates seven variable granule attributes. Analyses of the interdependence of these attributes indicate four key pigmentation characteristics, relatively independent of each other and determining variation of subordinate attributes;

nature of granule color, and setting limits on granule number, size and shape; granule size, determining shape and color intensity; granular clumping, damping the upper limit of granule number; and level of pigmentation, a complex determining medullary number, degree of pigmentation lag, cortical number, tendency to distal arrangement and black-fuscous granule size. Changes in internal environment along the hair axis affect the pigmentation level somewhat. The agouti series genes control a trigger mechanism which shifts reversibly the nature of granule color. The effect of the albino series is quantitative change in level of pigmentation. The major effect of the brown genes is on nature of granule color in eumelanotic regions. Two key pigmentation characteristics, size of granules in eumelanotic regions, and level of pigmentation are affected by the pink-eye alleles. The main effects of dilute substitution are production of granular clumps and reduction of cortical pigment. All this evidence supports WRIGHT'S concept of the genes of each allelic series causing quantitative changes in "the strength of a single primary reaction."

RUSSELL, W. L., and PATRICIA M. DOUGLASS, Roscoe B. Jackson Memorial Laboratory, Bar Harbor, Maine: *Ovarian transplantation as a tool in genetic research.*——Recently reported experiments have shown that offspring can be obtained from pure strain mouse ovaries transplanted to hybrids. This method is being used to study the maternal environment. Preliminary results indicate that number of lumbar vertebrae in an inbred strain is affected when the animals develop in hybrid mothers. Other experiments have demonstrated that offspring can be produced from unborn mothers by transplanting embryonic ovaries to adult females. This technique offers a variety of possibilities in several fields of research. For example, by transplanting ovaries from abnormal embryos of a presumed dominant lethal type it is proving possible to determine the genetics of these embryos by direct breeding tests.

SCHULTZ, JACK, Lankenau Hospital Research Institute, Philadelphia, Penn.: *The analysis of chromomere patterns in man.*——By the application of smear techniques to cells stripped from the seminiferous tubules of the testis, it is possible to obtain acceptable preparations in which the pachytene chromosomes of man are freed from the nuclei and can be studied in detail. At the present time preparations have been obtained from 22 individuals. The individual chromosomes have their distinctive patterns, although difficulties arise due to the association of the heterochromatic regions that occur on the various chromosomes, as well as to the possibility of terminal associations. The sex chromosomes are associated with a nucleolar mass; there is another nucleolar region associated with one of the longer chromosomes. The present data give the expectation of four nucleoli in the diploid complement in man, when there is no nucleolar fusion; any higher number is therefore due to polyploidy in the affected cell. A detailed study of the chromomere pattern of the nucleolar chromosomes has been made (SCHULTZ and ST. LAWERNCE) to determine whether the possibility of chromomere maps in man can be realized. Sufficient

constancy has been found both within and between individuals to establish the regular occurrence of the nucleolus between two pairs of heavy chromomeres, heterochromatic regions. The "arms" on either side of the nucleolus are composed of ten and 12 chromomeres, respectively, in distinguishable patterns. In the population we have studied so far, no inversions of this chromosome have been detected, although all the larger types could have been seen had they occurred. Studies of the other chromosomes are under way, to test further the possible extension of cytogenetic methods in human heredity.

SCOTT, J. P., Roscoe B. Jackson Memorial Laboratory, Bar Harbor, Maine: *"Normal" reactions to the frustration of fighting in an inbred strain of mice.* (A motion picture).——Excluding such cases as audiogenic seizures, most reports indicate that training and frustration are important causes of experimental neurosis in animals. If training is an essential factor, the frustration of inexperienced animals should produce adaptive or mal-adaptive behavior rather than non-adaptive behavior. The fighting of inexperienced male mice of the C_{57} strain was frustrated by matching them with superior opponents, the result being adjustment by running. In a second experiment this latter adjustment was frustrated by decreasing the size of the pen, the result being a shift in adjustment to a "defensive posture." In both cases there was a change in the type of behavior in response to frustration, and in both cases the behavior appeared to be obviously adaptive or partially adaptive. It may therefore be concluded that training is probably an essential factor in the production of abnormal (non-adaptive) behavior in this situation and in this particular strain, and that the primary effects of frustration by itself are normal behavior. The behavior described may be used as a standard for the study of abnormal behavior in comparable situations.

SEARS, E. R., University of Missouri, Columbia, Mo.: *Isochromosomes and telocentrics in Triticum vulgare.*——For the speltoid (IXth) chromosome of common wheat, isochromosomes and telocentrics involving the long arm constituted 7.0 and 10.8 percent, respectively, of the 157 detected chromosomes transmitted through 785 eggs from monosomic IX plants. More than half the products of misdivision could presumably not be identified as such, being normal chromosomes, complete deficiencies, or isochromosomes or telocentrics for the genetically unmarked short arm. Assuming that elimination of misdivision products is at approximately the same rate as for normally divided monosome IX, the data are in accord with the cytological observation of 36 percent misdivision (in 64 microsporocytes). Univalent isochromosomes and telocentrics have shown no striking differences from normal monosome IX in their transmission and misdivision frequencies. In somatic stages telocentrics tend occasionally to be lost or to give rise to isochromosomes, while isochromosomes tend with lower frequency to be entirely lost, to lose one arm, or to add a telocentric chromosome. Changes other than loss or addition of entire arms

also occur. A telocentric gave rise (apparently at meiosis) to a chromosome with two arms, of which the longer was of less than normal length, and the shorter was homologous with a portion of the longer. An isochromosome (for chromosome VII) gave rise in somatic tissue to a dicentric chromosome, with one centromere subterminal and the other apparently terminal on the short arm. This dicentric chromosome usually passed unchanged through meiotic and mitotic divisions.

SHULL, A. FRANKLIN, University of Michigan, Ann Arbor. Mich.: *Extent of genetic differences in male genitalia between Hippodamia quinquesignata and H. convergens (lady beetles).*——Because of the importance attached by taxonomists to male genitalia in insects as specific characters, and the theory that genitalia provide reproductive isolation of species, their genetic basis is significant. Two species of Hippodamia differ quantitatively in width of sipho and height of the flaps it bears, and qualitatively in presence or absence of an aedeagal keel. In a species cross the sipho and flaps are intermediate, and series of backcrosses rapidly approach the species to which they are crossed back. The keel is present until a certain genealogical nearness to the species which lacks it is reached; then it is poorly developed in some individuals, wanting in others. These results suggest a group of similar nondominant and (with respect to the quantitative characters) epistatic genes. The distinctions between the species appear to rest on two to five differentiating pairs of genes, with a clear possibility that the number may be three for each character. Positive correlation exists between the quantitative characters wherever genetic uniformity is expected, reduced or negative correlation where genetic variability is probable. This suggests that the genetic basis of these two characters is partly common to them. Males with and without the keel in one family differed in a way indicating that the keel may have a genetic basis partly common to the sipho and flaps. Correlation in this family suggests that the number of genes involved may be greater than the other results require. In any case, the species differences need not be very complex.

SNYDER, LAURENCE H., P. C. CLARK, and MURRY D. SCHONFELD, Ohio State University and the State Hospital, Columbus, Ohio: *The Rh factor and mental disorders.*——The fact that the frequency of clinically diagnosed cases of erythroblastosis has never approached the theoretical frequency to be expected on the basis of random matings has led to the search for other manifestations of the immunization. Recently YANNETT and LIEBERMAN, and SNYDER, SCHONFELD and OFFERMAN showed that feeblemindedness may be such a manifestation. From this premise we have started an analysis of the *Rh* factor in patients with various mental disorders, and their mothers. This paper is a progress report on the results with dementia praecox. Among 49 mothers of dementia praecox patients, seven (14.3 percent) were *Rh* negative. Among the 50 patients, four (8 percent were *Rh* positive children of *Rh* nega-

tive mothers. These results are not significantly different from expectation, and it appears that dementia praecox cannot be considered the result of immunization by the *Rh* antigen.

SONNEBORN, T. M., Indiana University, Bloomington, Indiana: *Inert nuclei inactivity of micronuclear genes in variety 4 of Paramecium aurelia.*— To obtain animals having different genes in the micronucleus and macronucleus of the same cell, k/k non-killers were crossed to K/K killers, and macronuclear regeneration was induced in the non-killer exconjugants that received an excess of kappa (the cytoplasmic factor for killer) from their killer mates. These exconjugants have syncarya of constitution K/k and the new macronuclei produced by the syncarya have the same genotype. From each exconjugant some lines of descent have their macronuclei produced in this way and others have instead macronuclei regenerated from pieces of the disintegrated preconjugation macronucleus (genotype k/k). Both sorts of lines have K/k micronuclei. Lines with K/k new macronuclei become killers; lines with k/k regenerated macronuclei remain non-killers. Hence the K gene in the micronucleus is unable to control the killer character in the presence of adequate kappa, but the same gene in the macronucleus can. To test whether kappa might be maintained in or by K in the micronucleus, the non-killers were induced to undergo normal autogamy, so as to form some macronuclei of genotype K/K. The killer character failed to appear in any of the progeny in this or subsequent autogamies. Hence, kappa had been lost, and the K gene in the micronucleus is unable to control or maintain kappa.

STALKER, HARRISON D., and H. L. CARSON, Washington University, St. Louis, Mo.: *Geographical variation in the morphology of Drosophila robusta.*— Recently collected strains from Texas, Alabama, Tennessee, Missouri, Illinois, Pennsylvania, and New York were reared under uniform conditions and the following ratios were determined for females: thorax length/head width; thorax length/femur length; wing length/thorax length; and wing length/wing width. Although there is overlapping between all strains for each of these ratios, statistically significant differences are noted in many cases, particularly between northern and southern strains. In general, northern strains show significantly higher values for thorax length/head width; wing length/thorax length and wing length/wing width, while southern strains show significantly higher values for thorax length/femur length. The F_1 of Austin, Texas × Rochester, N. Y., and Austin × Glen Ellyn, Illinois, and their reciprocals show ratios which fall between those of the parent strains except in the case of wing length/thorax length, where the F_1 of Austin × Rochester and the reciprocal both show ratios as high as or higher than either parent strain. All F_1 hybrids are larger and apparently more fertile than their parents. Further studies are in progress.

STERN, CURT, University of Rochester, Rochester, New York: *The phenotypes of heterozygotes between alleles in normal and translocated positions.*—— In *Drosophila melanogaster*, at 26°C the normal allele, $+^c$ of the mutant cubitus interruptus (*ci*) in the fourth chromosome is dominant over *ci*. If, as a result of irradiation with X-rays, the position of $+^c$ is changed, the heterozygote between the "position allele," $R(+)$ and *ci* frequently shows vein interruption of the *ci* type. Nineteen different $R(+)$ alleles in heterozygotes $R(+)/ci$, were tested and the mean degree of interruption compared with that of the sibs of the homozygous constitution *ci/ci*. Thirteen $R(+)$ alleles gave heterozygous $R(+)/ci$ phenotypes whose mean lie between normality and typical *ci/ci*, two $R(+)$ alleles produced phenotypes similar to *ci/ci*, and four gave phenotypes more extreme than *ci/ci*. Since these four $R(+)$ alleles in hemizygous and, as far as viable, in homozygous constitution produce uninterrupted normal phenotypes, the heterozygotes offer the unique phenomenon of a more extreme phenotype than either homozygote. However, the "addition" of another *ci* allele, resulting in triplo-4 $R(+)/ci/ci$, causes a shift back to less abnormality than *ci/ci*. The significance of these facts for interpretations of the position effect will be discussed.

STILES, KARL A., Michigan State College, East Lansing, Mich.: *The inheritance of spaced teeth.*——The inheritance of spaced teeth has been studied through five generations including a total of 55 individuals. The conspicuous spacing of the teeth in this family is limited to the central and lateral incisors of the upper jaw. The trait appears more often and is more marked in the males than in the females. Out of the 43 genetically related persons 27 possessed spaced upper incisors. Although the character appears in all five generations of the pedigree, there were two instances in which the trait did not appear in the parents but was exhibited by their children. Thus it appears that the character is not inherited as a simple Mendelian dominant, but rather an irregular dominance is indicated.

TAN, C. C., Department of Biology, National University of Chekiang, Hangchow, China, and Department of Zoology, Columbia University, New York City: *Mosaic dominance in the inheritance of the color patterns in the lady-bird beetle, Harmonia axyridis.*——*Harmonia axyridis* is a species of predaceous lady-bird beetle, belonging to the family Coccinellidae. Its distribution extends from southern Siberia (Altai Mountains) to Manchuria, Korea, Japan and China. The color patterns on the elytra, and also on the pronotum, of this species are extraordinarily variable, so much so that some of the color patterns have been described by the old school of taxonomic entomologists as belonging not only to several species but also in fact to different genera. Of no less than two hundred color forms known from different localities, each is found in only a part of the geographic distribution area of the species.

The inheritance of the elytral color patterns of the beetle, based on the material collected in Meitan of Kweichow province, southwestern part of China, can be accounted for by assuming 12 alleles of an autosomal locus. The color patterns and the alleles responsible for them are *succinea* (*s*), *conspicua*-1 (S^{C1}), *conspicua*-2 (S^{C2}), *transversifascia*-3 (S^{T3}), *transversifascia*-1 (S^{T1}), *equicolor* (S^{E}), *spectabilis*-1 (S^{S1}), *spectabilis*-2 (S^{S2}), *intermedia* (S^{I}), *aulica*-2 (S^{A2}), *aulica*-1 (S^{A1}), and *tripunctata* (S^{R}).

Succinea homozygotes (*ss*) have yellow elytra with a varying number of black spots (0–19). The number and size of the spots present in homozygous *succinea* depends upon the temperature during the pupal development. All other variants of the elytral color patterns have a black background with yellow or orange spots.

The crosses between *succinea* homozygotes and each of the 11 other variants give rise to heterozygotes in which the *succinea* characters are recessive except that the characteristic spots of the *succinea* parent may be found on the yellow areas.

Heterozygotes involving alleles other than *succinea* may be classified into two categories—namely, inclusive combinations and overlapping combinations. In the former, which result from two alleles such that the black pigmented area characteristic of one in homozygous condition is included in that characteristic of the other allele in homozygous condition, the pattern of the heterozygotes cannot be distinguished from that of the homozygotes having the more extensive black pigmented area. In the case of overlapping heterozygotes, which are composed of two alleles such that the black pigmented area of the pattern characteristic of one allele in homozygous condition overlaps in the region of distribution of that characteristic of the other allele in homozygous condition, the pattern of the heterozygotes can invariably be distinguished from the patterns of either parental homozygous type.

The expression of the color pattern in the heterozygotes conforms to the rule of mosaic dominance, which states that any portion of the elytra which has black pigment in the homozygotes for a given allele will have black pigment also in the heterozygotes in which that allele is present. The only exceptional individual which seems to break this rule shows the mosaic effect of three alleles—namely, S^{E}, S^{R}, and *s*. This individual might have been a trisomic.

The rule of mosaic dominance has been found equally applicable to the heterozygotes carrying three alleles, which have been studied by HOSHINO (1940, Journal of Genetics)—namely, *axyridis* (S^{X}), *transversifascia*-2 (S^{T2}), and *forficula* (S^{F}).

The remarkable precision of the mosaic dominance phenomenon that is found in the expression of the color patterns of the heterozygotes between any two of the 15 known alleles affords interesting material for further investigations in certain aspects of both evolutionary and developmental genetics.

VILLEE, CLAUDE E., Harvard Medical School, Boston, Mass.: *Some de-*

veloping effects of irradiating Drosophila.——Canton, aristapedia, aristapedia-Bridges, bithorax, proboscipedia, dachs, dachsous, four-jointed, erupt, and double antennae flies were irradiated as larvae, prepupae, or pupae with dosages ranging from 1906 to 7000 r units at several different intensities. One effect, found with all except the lowest dosages used, was a marked retardation of pupation. Larvae 100 hours old when irradiated, which should have pupated within 24 hours, remained alive as larvae for periods of eight to 12 days before pupating; most of these died shortly after pupating. The stage in development irradiated was found to be extremely important in determining the type of abnormality produced, and the phenocopies obtained were similar in many respects to those obtained by WADDINGTON (1942). In contrast to WADDINGTON'S results, different phenocopies were obtained when different stocks were given identical irradiations at the same stage of development. In addition to the usual phenocopies, the absence or malformation of some structure, examples of abnormal histogenesis were found; leg-like antennae, split eyes, palps in eyes, prothoracic palps or wings, doubled wings, and others

VILLEE, CLAUDE E., Harvard Medical School, Boston, Mass.: *Dosage and intensity relations in the irradiation of Drosophila.*——Larvae, prepupae and pupae of several stocks of *Drosophila melanogaster* were irradiated with a variety of intensities and total dosages of X-rays. A marked difference was found in the results of irradiations of about the same dosage given at different intensities. 6925 r units, given at 5540 r units per minute, caused a much higher lethality both before and after pupation than 7000 r units at 78 r units per minute. A similar higher lethality resulted from 5540 r units, given at 5540 r units per minute, than from 5460 r units at 78 r units per minute. The lethal effect does not depend simply upon the total amount of irradiation, but to a large extent upon the intensity of X-rays.

WARMKE, H. E., Institute of Tropical Agriculture, Mayaquez, Puerto Rico: *An analysis of male development in Melandrium by means of Y chromosome deficiencies.*——Present evidence, based on a study of Y chromosome deficiencies of various types and lengths in 2A XXY stocks, indicates the presence of at least three genes or gene complexes in the Y of Melandrium that operate in the development of maleness. First, there is one near the centromere which *initiates male development.* When only this proximal segment of the Y is present stamens start, but their development is arrested shortly after meiosis; when this segment is absent no male structures are initiated. Second, there is a gene or group of associated genes near the end of the differential arm of the Y which completes *male development.* When the differential arm is present (in addition to the proximal segment) full male development results, but when the terminal one-fourth or more of the arm is removed male development is incomplete. Third, there is a gene or region in the terminal one-fourth of the homologous arm which *supresses femaleness.* When the entire Y chromosome is present (in addition to two X chromosomes) female structures in the

hermaphrodite are poorly developed, but when the tip of the homologous arm is absent, female development is essentially complete. In addition, duplications of certain of these regions of the Y chromosome apparently cannot substitute for deficiencies of others.

WEATHERWAX, PAUL, Indiana University, Bloomington, Indiana: *Corn for morphological and genetic work.*——During the past year a collection has been made of varieties of corn grown, principally by the Indians in Arizona, New Mexico, Mexico, Central America, Ecuador, and Peru. These exhibit a wide range of endosperm and pericarp types and ear structures, with the implication of accompanying variations in plant characters. Selected specimens will be exhibited. Future work on this material is planned, and suggestions will be appreciated.

WHARTON, LINDA T., University of Texas, Austin, Texas: *Interspecific hybridization in the melanopalpa subgroup of the repleta complex.*——Several interspecific hybridizations have been reported among the members of the *melanopalpa* subgroup of the *repleta* complex which is comprised of a large number of closely related Drosophila species. Three species, *D. canapalpa*, *D. melanopalpa*, and *D. neorepleta* have been hybridized with strains of *D. repleta* where the latter species was used as the male parent. *Drosophila neorepleta*, *D. melanopalpa*, and *D. canapalpa* can be hybridized with each other in reciprocal crosses. Two factors which have rendered this subgroup difficult to work with are a high degree of initial sexual isolation and the small number of chromosome rearrangements in the salivary chromosomes of the hybrids. An additional member of this subgroup has been described and designated as *Drosophila limensis* (PAVAN and PATTERSON, unpublished description). The new species differs from all other members of the subgroup with regard to metaphase chromosome configuration, having one pair of long rods (the X chromosomes), three pairs of medium length rods and two pairs of short rods. The Y chromosome is quite short. Four types of metaphase chromosome configurations are now known for this subgroup. In the interspecific hybridization of *D. limensis*, the strong primary isolation encountered in other hybrid combinations is lacking. Furthermore, although synapsis is effective in the salivary chromosomes of hybrid larvae, *D. limensis* differs considerably from the other species in gene order. Since *D. limensis* links the members of the *melanopalpa* subgroup so closely, a more critical analysis of the genetic and phylogenetic relationships of the species is possible.

WHITING, ANNA R., University of Pennsylvania, Philadelphia, Penn.: *Immaternate males and high visible mutation rate from eggs irradiated in prophase I*——Unmated wild type females of Habrobracon were heavily X-rayed and mated to recessive untreated males. Surviving progeny (about 10 percent for 28,000 r) were from eggs irradiated in prophase I. They consisted of the expected wild type, haploid impaternate males and diploid biparental hetero-

zygous females and, in addition, recessive haploid immaternate (androgenetic) males. The last resembled their fathers in every respect, were fully fertile and non-mosaic. Percentages of androgenesis (immaternate ♂♂×100/immaternate ♂♂+♀♀) were 4.21 (15/356) for 28,000 r, 23.06 (3/13) (corrected 13.36) for 29,300 r and 12.50 (1/8) for 42,000 r. Correction is applied to second group because crosses made were two-allele and therefore 50 percent of zygotes should be diploid males and inviable. Production of normal individuals from untreated sperm in heavily irradiated cytoplasm strengthens views that X-ray injury is directly chromosomal. The heterozygous females were bred unmated. 252 tested from 28,000 r treatment produced seven or more sons. Among these, heterozygosis for visible mutations was 12.69 percent. Tests of four from 29,300 r yielded no mutations and four from 42,000 r yielded one. These results suggest possibility of obtaining readily dose-action curves for visibles for comparison with those already available for lethals in this material.

WITKIN, EVELYN M., Carnegie Institution, Cold Spring Harbor, New York and Columbia University, New York: *A case of inherited resistance to radiation in bacteria.*——When a sample of Strain B of *Escherichia coli* is exposed to ultra-violet radiation, the last survivors prove to be relatively resistant to both ultra-violet and X-rays. The resistance is stable and heritable.—Survival curves for the radiation-resistant strain at various doses of ultra-violet differ markedly in shape from similar curves with X-rays. Survival curves for the normal strain with X-rays and ultra-violet are identical in shape.—The lag phase of the resistant strain in broth is only one-half the length of the normal lag phase. This apparent selective advantage disappears, however, when the two strains are mixed and incubated together for some time—the lag phase of the resistant strain becomes the same as that of the normal strain.—Microscopic examination of ultra-violet irradiated cells of the normal and resistant strains reveals striking differences. At low doses, normal cells elongate for many hours without dividing while resistant cells begin to divide soon after treatment. This difference is the basis of a technique for the accurate determination of the number of resistant cells in a sample, without which genetic analysis would be very difficult.—Resistance to radiation is in this case a mutation arising spontaneously in the bacterial culture, independently of treatment with radiation. This was demonstrated by following directly the increase with time of the proportion of resistants in a normal culture.

WOOLLEY, GEORGE W., Roscoe B. Jackson Memorial Laboratory, Bar Harbor, Maine: *A study of the pirouette mutation in the mouse.* (A motion picture.)

ZAMENHOF, STEPHEN, 620 West 115th St., New York 25, N. Y.: *Studies on bacterial mutability. The time of appearance of the mutant.*——This study deals with the problem of the well known but hitherto unexplained delay in the occurrence of the bacterial mutants. To eliminate the trivial part of this delay

—namely, the delay in the production of medium preferential for the mutant—the work was done on citrate unstable strains of *B. coli* (Parr), using citrate media which are always strongly preferential for the citrate+mutant. To make possible the quantitative study, the experiments were performed on a large number of Koser broth cultures. The following facts were established: (1) Given the adequate number of mother cells there is no delay in the occurrence of the first mutant cell: the mutant may appear as early as within ten hours after inoculation—that is among young and normal cells; in that respect these bacterial mutations may resemble the mutations in higher organisms. (2) The reason for the delay in the detection of this mutant cell has been found in the slow multiplication of the mutant and its early descendants in the mother culture; this slowness is probably caused by the poisoning by the thermolabile inhibitory factor found to be produced by the mother cells. If the poisoned mutant cells are transferred into the fresh medium, they multiply fast like normal citr.+cells, after the usual short lag period. Similar but less accentuated delay due to poisoning has been found in the normal citr.+cells if they are inoculated into 12 hours' old or older citr.−culture. (3) One pure mother strain may give rise to the entire gamut of mutants differing by the degree of citrate utilization and probably also by the degree of their resistance to inhibitory factors.

SUMMARIES FROM FORTHCOMING PAPERS

STURTEVANT, A. H., *On the dot chromosomes of Drosophila repleta and D. hydei.*—Received December 3, 1945.—An X-ray induced dominant mutant in *Drosophila repleta* is probably associated with a translocation between the dot and another autosome. An aneuploid type produced by this translocation is interpreted as being haplo-dot. A type phenotypically the opposite (as compared to wild type) is produced by *repleta-neorepleta* hybrids, and is interpreted as triplo-dot. Triplo-dot×triplo-dot gives a more extreme type, probably tetra-dot. Haplo-dot is dark-colored, sterile, and has blunt but not Minute bristles; triplo-dot is light-colored and fertile; tetra-dot is still lighter and is sterile. In *D. hydei* triplo-dot was detected by the use of the mutant Extended, and its composition was verified by cytological study. Here also triplo-dot is lighter in color than wild type. Preferential segregation occurs in triplo-dot *hydei;* it is more evident in males than in females. A discussion of the available genetic data on the dot chromosome leads to the conclusion that it is similar in its properties in all the species of the genus studied, but among these is perhaps most different in *repleta* and *hydei.*

DOBZHANSKY, TH., *Genetics of natural populations. XIII. Recombination and variability in populations of Drosophila pseudoobscura.*—Received December 5, 1945.—Three wild second chromosomes, derived from the population of *Drosophila pseudoobscura* of Mount San Jacinto, California, were chosen as material for experiments. One of these chromosomes is remarkable because individuals homozygous for it are highly sensitive to environmental influences: they survive nearly normally at 16½°, are semilethal at 21°, and completely lethal at 25½°C. Homozygotes for the other two chromosomes have viabilities about equal to those of the respective heterozygotes.

By means of appropriate crosses, females were produced that were heterozygous for any two of the above three chromosomes. The eggs of these females contained chromosomes some of which were identical with the ancestral ones (non-crossovers) and some were new (crossovers). A sample of these chromosomes was analyzed for their effects in homozygous condition. A great variety of chromosomes with different properties were thus obtained. The viability of the homozygotes for some of the chromosomes is equal to or even slightly superior to that of the respective heterozygotes; other chromosomes act as recessive deleterious modifiers, still others as semilethals, and finally some act as complete lethals. Homozygotes for some chromosomes are relatively more viable than heterozygotes in crowded cultures, others are favored in cultures with low population densities, and still others are not sensitive to population density variations. Many of the chromosomes produce varying degrees of retardation of the development of the homo- as compared to the heterozygotes.

Particularly interesting is the appearance of "synthetic" lethal and semilethal chromosomes, which arise through crossing over between chromosomes lacking these properties. One chromosome has a dominant effect on the de-

velopment rate of its carriers; no such effects were present in the ancestral chromosomes. At least two chromosomes have "synthetic" effects on the visible morphology of the flies.

It is concluded that chromosomes in natural populations of *Drosophila pseudoobscura* differ in complexes of linked genes most of which have small individual effect. These gene complexes afford a great store of potential genetic variability which may be released by crossing over and recombination, and then utilized for evolutionary changes. Genes with large individual effects also occur in wild chromosomes.

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